

The Journal of Laboratory and Clinical Medicine

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1. Reichstein and Euw—*Helv. Chim. Acta* 21:1197, 1938.
2. Thorn, et al—*Am. Jl. Med. Sciences* 197:718, 1939; *Jl. Clin. Invest.* 18:449, 1939.

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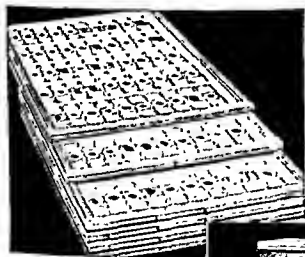
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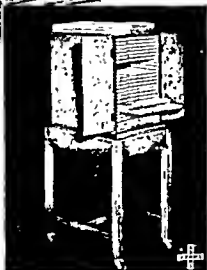
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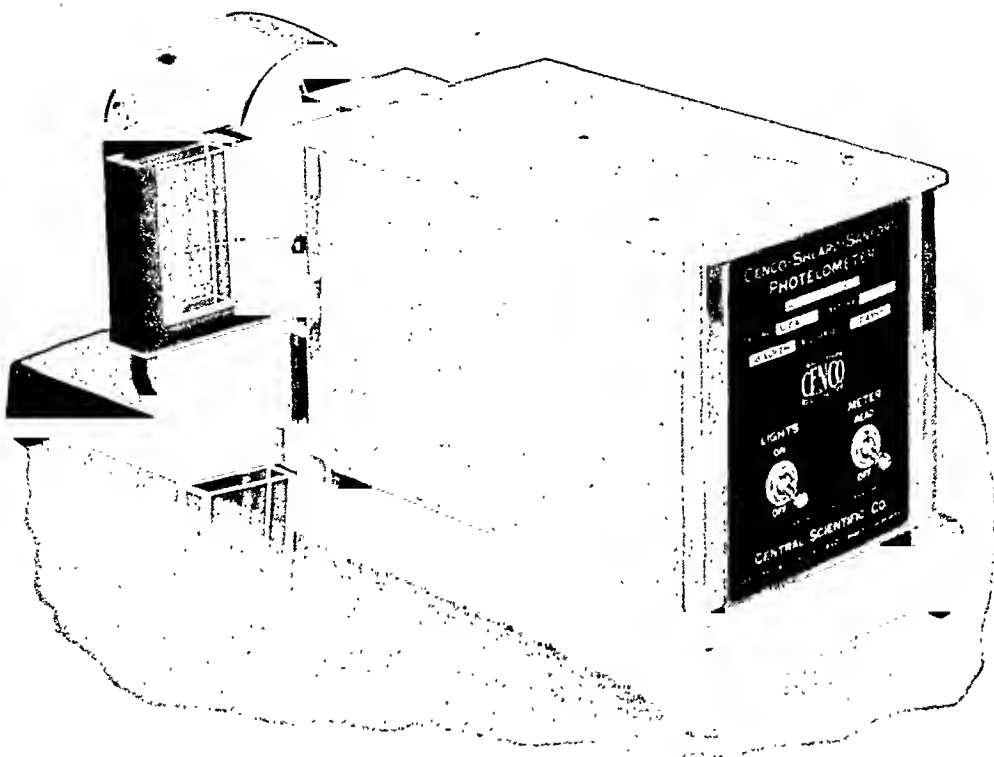
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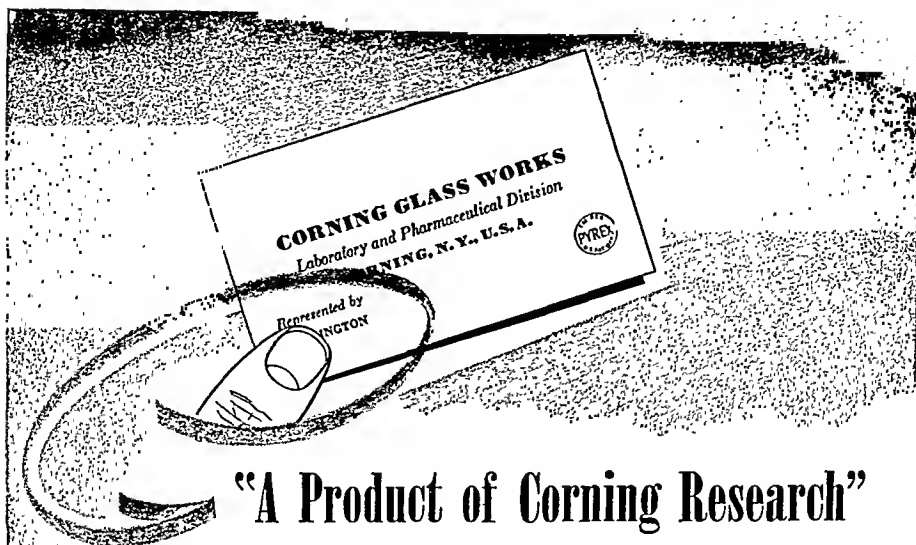
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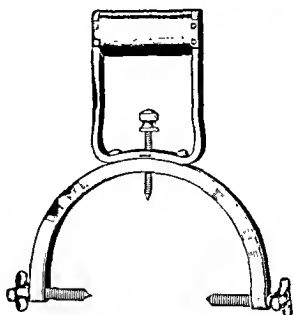
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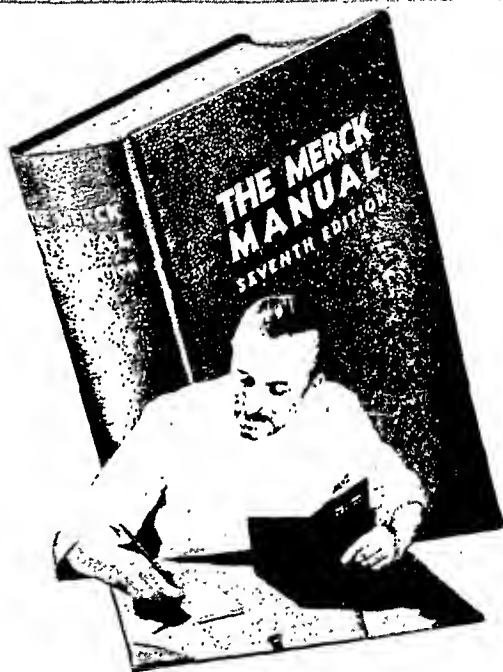
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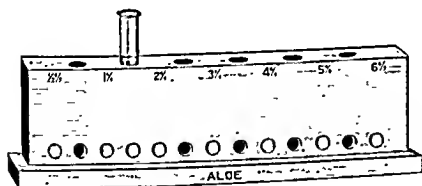
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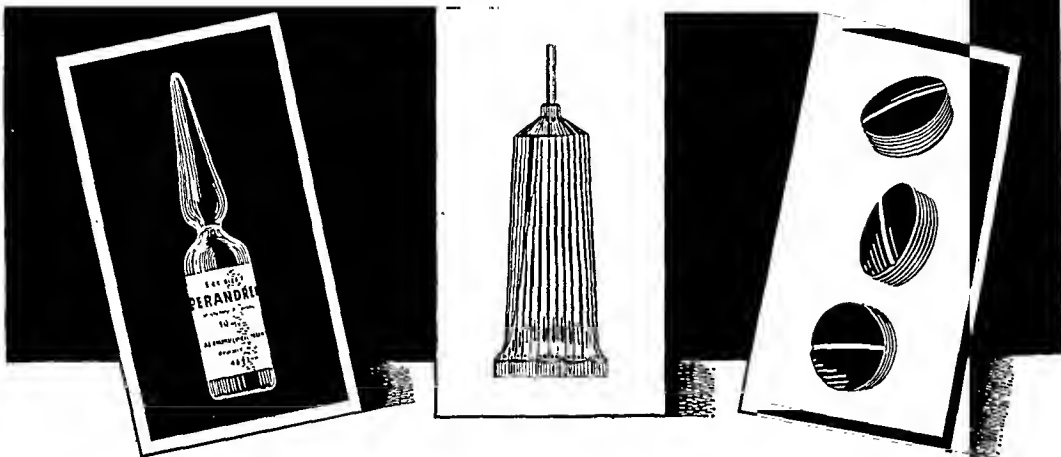
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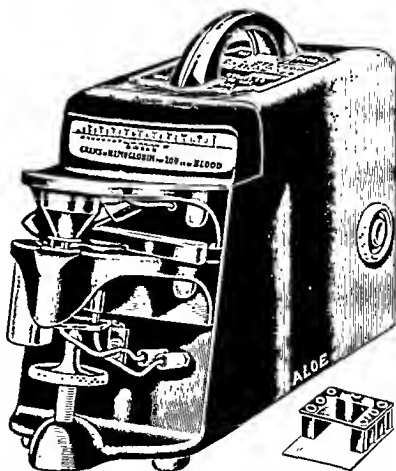
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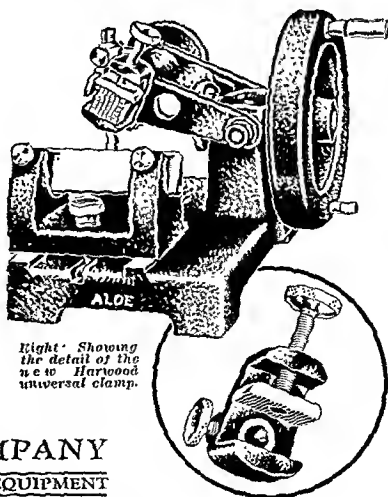
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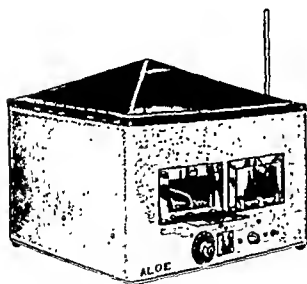
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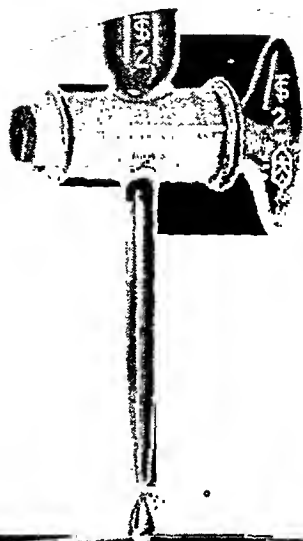
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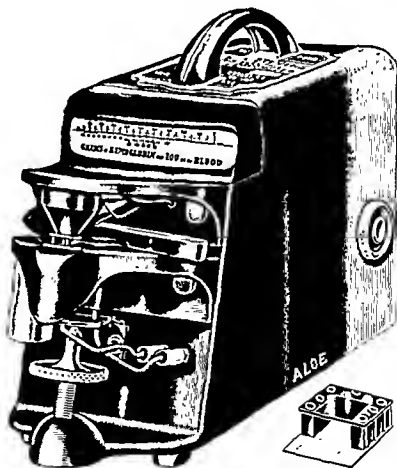
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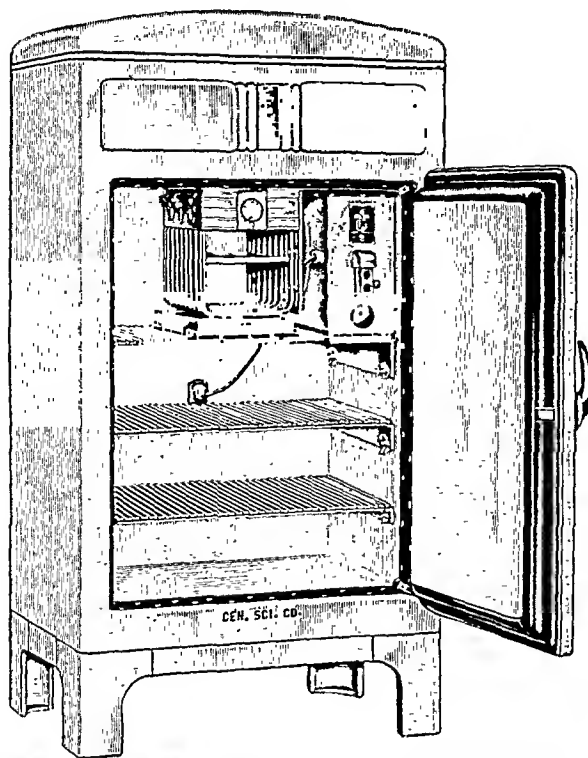
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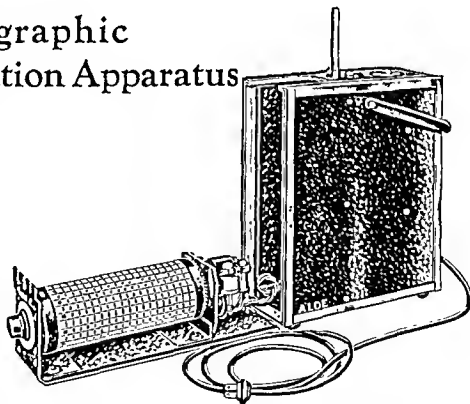
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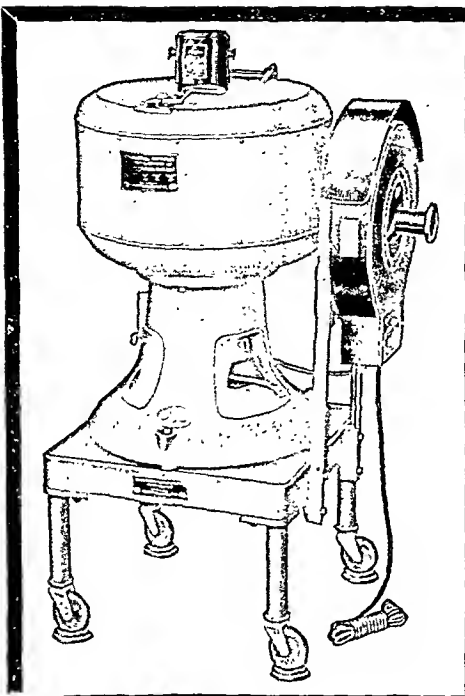


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To a Golden Anniversary Number

W. T. V.



VICTOR C. VAUGHAN



DENNIS E. JACKSON

The Journal of Laboratory and Clinical Medicine

VOL. 26

OCTOBER, 1940

No. 1

THE SILVER ANNIVERSARY NUMBER

We Celebrate Our Silver Anniversary

The twenty-fifth volume of *THE JOURNAL OF LABORATORY AND CLINICAL MEDICINE* is completed. This issue inaugurates a second quarter century of service to medicine.

Twenty-five and more years ago no single medical periodical adequately filled the gap between laboratory research and the application in the field of clinical medicine of those discoveries coming from this research. This *JOURNAL* was inaugurated primarily to serve as the connecting link between the laboratory and clinical medicine. Clinical pathology was then in its infancy. Common interests developed with those who were applying laboratory methods to clinical investigation. The *JOURNAL* soon became the standard medium for presentation of advances in clinical pathology.

In the first volume the section on Laboratory Methods was written by the editors, as critical reviews. It soon became apparent that this department was the only available classified medium for the presentation of new laboratory techniques and apparatus. Contributions became so numerous that in the second volume the editors discontinued their review contributions.

With the appearance of Volume XI original contributions to the section on Laboratory Methods required so much space that it was deemed advisable to inaugurate an additional department, Selected Abstracts and Reviews. With this, the *JOURNAL* could still call the attention of the readers to new advances.

The Editorial Section continued with longer reviews, but as the *JOURNAL* grew, the volume of good material submitted was so large that the interval between the time of acceptance and the date of publication became too long. To provide more space editorials were discontinued in Volume XXI.

Major emphasis through the twenty-five years has been on laboratory medicine. At the same time, to retain broad perspective, a reasonable proportion of purely clinical articles have been regularly accepted. As a rule, contributions with direct clinical application have received precedence, although a number which might be classed under pure research have been published, especially when

the editors have recognized the possibility of future use in clinical laboratory medicine. In this way the JOURNAL has helped to facilitate the application of pure research to clinical problems.

The JOURNAL has served another function, important for medical progress. From time to time it has opened its pages to new medical societies whose fields of interest combined the laboratory and the clinic, that they might have a medium for presentation of their transactions until they could organize and support their own periodicals. This service has been given to The American Society of Clinical Pathologists, The American Association for the Study of Allergy, and The American Rheumatism Association.

The editors in celebration of the JOURNAL's twenty-five years of service, believe that, instead of reviews of medical progress during that period, a symposium discussing most recent developments and their probable future implications would be more consistent with the past and present policy of facilitating the progress of research. This Anniversary Issue looks forward, not backward. For this reason, it has seemed appropriate to celebrate the event at the inauguration of the second quarter century, in Volume XXVI, rather than at the end of Volume XXV.

The editors express their deep appreciation to the authors who have contributed to the Anniversary Number. They hope that their readers will derive as much satisfaction from perusal of these pages as they have in their preparation. They believe that the subjects discussed will become increasingly important. Special effort has been made to avoid repetition of discussions which are readily available elsewhere. This applies, for example, to the sulfonamide compounds.

The make-up of the JOURNAL has been altered from time to time to provide for needs as they have arisen. A journal whose function is to mirror progress should not be static; it should experiment with new methods of presenting subject matter, with full realization that some of the trials may be unnecessary or unsatisfactory. In this event, there will be no hesitancy in altering or abolishing them.

The commencement of a second quarter century of publication is an appropriate time to put some of these tentative ideas into effect. To this end the JOURNAL announces certain additions to its departments.

Clinical chemistry has assumed steadily increasing importance in laboratory medicine. Articles in this field have been scattered in the departments devoted to Clinical and Experimental Investigation and Laboratory Methods. It is believed that a separate department of Clinical Chemistry will focus interest in this field and will facilitate classification of the material presented. The new department of Clinical Chemistry will be edited by Dr. Victor C. Myers and Dr. Edward Muntwyler of Western Reserve University.

The number of letters discussing editorial reviews of medical progress has in the past made the editors deeply conscious of the interest which they have stimulated. It is regretted that space limitations required their discontinuance. Believing that progress reviews of this type serve a real function, the editors of the JOURNAL will again inaugurate reviews, but with a new, and it is hoped improved, method of approach.

The men best qualified to write progress reviews are those who are themselves conducting pioneer investigations in the fields. From time to time there will appear, therefore, as leading articles, invitation contributions on subjects analogous to those presented in this Anniversary Number, and written by outstanding investigators. If these adequately fill a need, they will be made regular sections of the JOURNAL.

The importance of good illustrations has been recognized from the outset. The JOURNAL's excellent photographic reproductions have been an outstanding feature. Not only has medical illustration become a specialty in its own right, but with the improvements of the last decade in precision photography, the physician often illustrates his own articles. Many have found photography a pleasant hobby and a useful one, since it can be used in connection with one's work.

The *Journal of the Biological Photographic Association* serves those deeply interested in scientific photography. Not limited to medicine, it covers all fields of science.

The editors are of the opinion that a small department of medical illustration, covering photographic and other methods, will be of sufficient concern to those readers now interested in photography, or to those who may become interested, to justify its establishment. This department, by broadening interest in the subject and providing an alternate medium for publication, will not compete with the *Journal of the Biological Photographic Association*, but will complement it and provide a classified department on other methods of medical illustration. It will be inaugurated in January under the editorship of Carl D. Clarke, Professor of Art as Applied to Medicine and Director of the Department of Art of the University of Maryland Medical School, who organized and first edited the *Journal of the Biological Photographic Association*.

During its existence the JOURNAL has grown from approximately 950 pages of Volume I to over 1,300 pages in Volume XXV. The new departments will require either the addition of about 400 pages or some limitation on the nature of subjects now considered appropriate for acceptance. Suggestions and constructive criticism on this point and on other subjects herein discussed, but particularly with regard to any subject matter in the last few volumes which may have been of interest to too small a proportion of the readers to justify its publication, are welcome.

The editor expresses his deep appreciation to all the writers who have contributed to the JOURNAL during his seventeen years of editorship, and to those whose earlier papers were accepted by the first editor; writers whose contributions have given life to the JOURNAL and enabled it to serve adequately as a functionary in the progress of medical knowledge.

WARREN T. VAUGHAN

THE AUTONOMIC NERVOUS SYSTEM CONSIDERED IN RELATION TO EXPERIMENTAL AND CLINICAL PHENOMENA*

DENNIS E. JACKSON, PH.D., M.D., CINCINNATI, OHIO

IN A MANNER characteristic of the history of our times a contemporary wag has defined an adult as one who has stopped growing at both ends but continues to grow in the middle. This conception of a generalized entity composed of two ends and a middle, in either division of which growth, or at least change, may occur, coincides very well with our present conception and knowledge of the autonomic nervous system. Within recent years many changes, and a good deal of growth, have occurred in our understanding of this system. But not infrequently a change which at first appeared as growth has ultimately been found to be merely an added source of confusion. And in many respects our knowledge of the involuntary nervous system is still in an infantile or even an embryonic state. Current medical literature contains hundreds of references to such subjects as renin, renin-activator, angiotonin, ischemin, sympathin E and sympathin I, and intermedin; the carotid and aortic bodies and the corresponding sinus structures; choline-esterase, amine oxidase (see "sulfosynthase"), adrenergic and cholinergic nerves, benzedrine, veritol, corbasil, "933F," prostigmine, paredrine, beta-erythroidine, and trasentin and trasentin-6H; failures frequently following surgery on the sympathetic nervous system because sensitization to adrenalin develops at the nerve endings; the remarkable and peculiar symptom complexes which occur in the presence of pheochromocytomata; desensitization to food proteins by prolonged administration of mechoyl; the strange and often depressing mental and physical manifestations resulting from disturbances of the hypothalamus and related structures—these and numerous other new and unexpected experimental or clinical observations all tend to reveal the vast territory which the autonomic nervous system is rapidly usurping in the field of modern medicine. The names "sympathetic," "autonomic," "involuntary," and "vegetative" are often (but not quite correctly) used synonymously in referring to this system.

Anatomically the autonomic nervous system is considered to consist of two parts, the true or thoracicolumbar sympathetics emerging from the eighth cervical, and the thoracic and lumbar (to the third or fifth) divisions of the spinal cord, and the parasympathetics which pass out from the central nervous system in the third, seventh, ninth, and tenth cranial nerves and from the second to the fourth sacral segments of the cord. These primary emerging fibers are preganglionic, and they pass through greater or lesser distances to communicate with the nerve cells of the postganglionic fibers. The postganglionic fibers are highly specialized both as regards their anatomic dis-

*From the Department of Pharmacology of the University of Cincinnati Medical College, Cincinnati.

tribution and their individual functions. All these fibers are efferent in character (see below for sensory counterparts).

Returning to the structural conception of two ends and a middle, we may note that centrally the autonomic nervous system primarily originates in nuclei in the hypothalamus and in the medulla. But these centers have numerous connections, often unknown or at best only vaguely surmised, with higher divisions of the brain or with other neighboring nervous mechanisms. Mystery surrounds much of the workings of the hypothalamus, but it undoubtedly exercises a dominant control over many, perhaps most, of the functions of the autonomic nervous system. Through the hypothalamus and medulla impulses from above permit mental stresses or strains or other psychologic states, generally entirely unconsciously, to exert their influences on the more distal portions of the autonomic nervous system. Drugs may affect all these regions and the pharmacologic interpretation of these reactions may be exceedingly difficult, and especially so if the effects are obscured, as would usually be the case, by more peripheral actions. If pathologic conditions in the hypothalamus, or in higher related structures, produce serious mental or physical changes, one can only conjecture as to how drugs may act on these structures in order to be therapeutically valuable to the patient.

In recent years, a vast amount of work has been done to explain the actions which take place at the peripheral endings, both of the preganglionic and of the postganglionic neurones. Already one Nobel prize has been granted for work in this connection. Perhaps there is still room for another. When impulses pass outward over preganglionic fibers, both true sympathetic and parasympathetic, these impulses arrive in due course at the endings of these neurones around the nerve cells of the postganglionic neurones. At this point each impulse is now presumed to cause the liberation of a minute quantity of acetylcholine which transmits the excitation to the next receptor, that is, the cell of the postganglionic neurone in this case. The liberated acetylcholine lasts for only the briefest instant of time, and then it is equally rapidly hydrolyzed by cholinesterase which is always present due to its wide distribution in the tissues. An exactly similar action is also believed to occur in striated muscles when nervous impulses pass out from the central nervous system over the cerebrospinal motor nerves to striated muscle nerve endings. All preganglionic nerve endings, and all parasympathetic nerves whose endings are thus stimulated by acetylcholine, are designated as cholinergic. If eserine in small quantity is applied to these endings previous to stimulation of the nerves, it is presumed to take up and destroy a certain amount of the esterase normally present. Thus, when a nerve impulse passes down the nerve fiber and liberates a charge of acetylcholine, the action of the latter will be prolonged as manifested in the muscle or gland cell by its increased or prolonged activity. In certain instances, such as the sweat glands, nerve fibers which anatomically belong to the thoracolumbar sympathetics are cholinergic in action and pharmacologically react as do the parasympathetic nerves.

For many years a queer problem was presented by the results following electrical stimulation of certain true sympathetic nerve fibers. In some cases

smooth muscle contracted, in others it relaxed. When adrenalin was found to produce similar results, it was surmised that there must be some difference in the chemical constitution of those muscles, yet no analyses could demonstrate this. A little later on a considerable shifting of viewpoints led to the almost universal use of adrenalin to detect the presence or absence of true sympathetic nerve endings. But the problem of contraction in some cases and inhibition in others still remained. Within recent years, however, a number of new conceptions have been advanced in the hope of explaining these phenomena. It is now presumed that impulses passing down the true sympathetic nerves (except those which are cholinergic in nature) cause the liberation of adrenalin (Dale) at the terminal endings of these fibers, and that this adrenalin transmits the excitation to the muscle or gland cell. Cannon and his co-workers have advanced the view that either adrenalin or some similar substance is liberated by the nerve impulses, and that this substance at once unites with one or the other of two substances (E and I) already present at the nerve endings to form sympathin E (effector) or sympathin I (inhibitor). If strong stimulation of the nerve leads to formation of excessive amounts of either sympathin E or sympathin I, then this may be carried away in the blood stream (or perfusion fluid) and produce corresponding results on other similarly innervated tissues. Nerves of this character are designated as adrenergic.

The method by which adrenalin is inactivated in the body has been a standing question. Gaddum and Kwiatkowski¹ (1938) have advanced the view that adrenalin is oxidized in the body by amine oxidase. As supporting evidence of this, they note that ephedrine increases the action of adrenalin and also that ephedrine inhibits the action of amine oxidase. This view corresponds to that of the action of choline-esterase and eserine as they affect the action of acetylcholine.

Opposed to this view, recent admirable work has been published by Richter² in which he finds that adrenalin is excreted in the urine in a conjugated (sulfate ester) form. This conjugation probably takes place largely in the liver, and while some adrenalin may be oxidized by amine oxidase, still conjugation is a more rapid process and is probably the method by which most of the adrenalin disappears from the body. The conjugation is effected by the "sulfosynthase" system which is responsible for the conjugation of sulfate with phenols in the body. The liver is particularly effective in removing both adrenalin and phenols from the circulating blood. Since polyphenols use up "sulfosynthase" in the process of conjugation, it might be expected that administration of polyphenols, together with adrenalin, would lead to an augmentation and prolongation of the action of the adrenalin. This has been shown to occur. In a general way, therefore, the interactions of eserine, choline-esterase, and acetylcholine in the cholinergic system would correspond to polyphenols, "sulfosynthase," and adrenalin in the adrenergic system.

A further interesting point has also been brought out by Richter. It has generally been stated that adrenalin is only slightly, if at all, active when

taken by stomach. Richter has found that adrenalin is active when administered orally and will produce, over an extended period, the usual actions of the drug, but in a less acute degree. As adrenalin is ordinarily taken by mouth, most of the drug is oxidized in the gut. This oxidation can be mainly avoided by taking the adrenalin (e.g., dose 15 mg. adrenalin) with 0.1 Gm. glycine and 10 c.c. of 1 per cent acetic acid in 50 c.c. of water; up to 70 per cent of the adrenalin thus taken has been recovered (in conjugated form) in the urine. No free adrenalin has been found in the urine, and the presence of the drug cannot be detected until the conjugated drug has been hydrolyzed by boiling (the urine) with sulfuric acid. After purification and proper chemical manipulations the recovered free adrenalin gives a positive reaction with as many as seven chemical and color tests and also produces inhibition of isolated strips of intestine. It has also been shown that corbasil, epinine, and d-adrenalin, as well as l-adrenalin (the natural form), are all eliminated in the urine in corresponding conjugated forms. In all these cases the derivatives formed are apparently the sulfate esters, and conjugation occurs on one of the phenolic hydroxyl groups. This method of inactivation of sympathomimetic amines probably holds good also for many other members of the group, but not for all.

It has been the usual custom to consider the autonomic nervous system as a purely and solely efferent or motor system. But it must be fully recognized that there is a sensory counterpart in most, if not in all, autonomic actions. The sensory impulses which initiate autonomic motor effects are transmitted centrally over sensory fibers belonging to the cerebrospinal system. These fibers have their sensory cells in the posterior root ganglia of the spinal nerves or, in the case of the cranial nerves, in the corresponding sensory ganglia on the course of these nerves, such as the Gasserian ganglion on the fifth nerve and the ganglion jugulare and the ganglion nodosum on the tenth nerve. Throughout life enormous numbers of sensory impulses must be constantly passing into the central nervous system from most of the organs and structures of the body. These impulses set up numerous reactions in the sympathetic nervous system that must very generally be considered as reflex in nature. Some of these reflexes may be fairly simple in character, but others are extraordinarily complicated. Clinically they may represent the nervous mechanisms involved in an endless variety of aches, pains, swellings, indigestion, cardiac irregularities, blood pressure changes, secretory disturbances, metabolic or temperature variations, etc.

It was observed by Dale many years ago that an injection of adrenalin, following a sufficient dose of ergotoxine intravenously, caused a fall in blood pressure instead of the usual rise. This was interpreted as being due to a paralysis of the vasomotor constrictor nerve endings by the ergotoxine, so that following this adrenalin could only stimulate the vasodilator endings which ergotoxine was presumed not to paralyze. Rothlin arrived at the conclusion that ergotoxine, at least in large doses and in some locations, had a paralyzing action on certain sympathetic inhibitory endings as well as upon the motor endings.

In recent years various observers have obtained different reactions in tissues by a number of different drugs when different anesthetics were used. In certain instances this phenomenon may be very striking; perhaps it has not been sufficiently appreciated in the older investigations of sympathomimetic drugs. A relatively new preparation, "933F" (F stands for Fournau who introduced the substance) piperidomethyl-3-benzodioxane, has been found to have a paralyzing action on sympathetic endings similar to that of large doses of ergotoxine. Small doses of ergotoxine stimulate motor sympathetic nerve endings like adrenalin, but large doses paralyze these endings.

Page³ and his co-workers have recently made a number of interesting observations on the vasopressor substance obtained from the kidney. This substance is called renin, but renin is not active until it has combined with an activator contained in the blood, and this combination (of the two substances) has been named angiotonin. Crystalline derivatives have been prepared from angiotonin which is an active vasopressor substance. It appears to act pharmacologically in a manner similar to that of pitressin. Another substance obtained from the kidney by Prinzmetal, Lewis, and Leo⁴ also has a vasopressor action and has been named by them ischemin. The chemical constitution or identity of these preparations has not yet been worked out. One can only speculate at the present as to what relationship, if any, these kidney products may bear to clinical hypertension. There appears to be some connection between the action of these preparations and the influences which the central nervous system exercises over chronic hypertension. The sympathetic nervous system is probably involved in the pathologic reactions necessary to the maintenance of this state. The work of Goldblatt and the recent experiments of Dock⁵ throw some light on the still obscure problems here involved.

Marraszi⁶ has recently advanced the view that adrenalin, or an adrenalin-like substance, produces inhibition when present in minute (but sufficient) quantities (generated at the inhibitory endings) in the synapses in sympathetic ganglia. This counteracts the stimulating effects of acetylcholine which is generated at the (motor) synapses in these ganglia when nerve impulses from the cord or brain reach the ganglia. Experimentally adrenalin (or sympathin) may be produced in the body in sufficient quantity to bring about this type of inhibition by stimulating the nerves to the adrenal glands or other sympathetically innervated organs.

A generation or so ago hopes were held out that a careful study of the chemical structure and formulas of drugs would ultimately lead to a rational explanation of how they act. In particular, a careful comparison of the formulas of related substances, such as the sympathomimetic amines like adrenalin, tyramine, epinine, etc., was expected to throw great light on the prediction of how any given compound would act in the body even without trying it out. This was a kind of biochemical conception of pharmacology. To what vagaries and delusions this line of reasoning may lead one is well illustrated by observations made by Koppanyi and his co-workers⁷ relative to the so-called vasomotor reversal produced by ergotoxine or ergotamine with reference to the rise or fall produced in the blood pressure by adrenalin when different

anesthetics are used. These authors found that ergotamine or ergotoxine in doses of 0.2 to 4.0 mg. per kilogram in cats usually produces vasomotor reversal to adrenalin under urethane, but not under barbiturate anesthesia. In the latter, the adrenalin pressor effects were usually increased in height and always in duration. Thus urethane anesthesia constitutes the most favorable, and barbiturate narcosis the most unfavorable, condition for the elicitation of vasomotor reversal to adrenalin. Under barbiturate narcosis there is a distinct synergism between epinephrine and the ergot alkaloids. Blood pressure rises produced by adrenalin are often higher after ergotoxine or ergotamine than from control injections, and are always more prolonged. Massive doses of ergotoxine or ergotamine in barbiturate narcosis diminish or abolish the rises from epinephrine without producing reversal. Physostigmine or prostigmine facilitates vasomotor reversal under urethane anesthesia, while large doses of atropine administered before the ergot alkaloids in urethanized animals prevent reversal by epinephrine. Kappany and his collaborators further find that since the action of a single effective dose of ergotamine lasts for several hours, it is possible to produce vasomotor reversal under urethane anesthesia, and after recovery from urethane to administer a short-acting barbiturate and produce in the same animal a potentiated, instead of a reversed, epinephrine effect. Conversely, it is possible to use a short-acting thiobarbiturate, such as pentothal, and obtain a potentiated epinephrine response following ergotamine, and after the recovery of the animal from the barbiturate, to give urethane and obtain a vasomotor reversal to adrenalin. Thus the difficulty of determining how or where a single member of a series of compounds acts is seen. The complications will increase when predictions for other members of the series, or for entirely different drugs which have similar pharmacologic actions, are attempted.

Barbiturates⁸ appear to depress or paralyze the vagus ganglia in the heart. Pilocarpine or acetylcholine may still slow the heart, while eserine may restore the excitability of the vagus but may not spontaneously slow the heart rate.

Actions mediated partly or entirely through the autonomic nervous system may be so extensive and involved that one cannot fathom all the changes that may be taking place at a given moment. But by special experiments in which only a small number of reactions may be studied at any one time, a fairly complete, composite picture may usually be built up to explain any special phenomenon as a whole.

One of the simpler autonomic reactions is that by which the systemic blood pressure is raised in the presence of asphyxia. The asphyxia may be produced in many ways, but experimentally stoppage of the respiration by electrical stimulation of a vagus nerve is perhaps the most common. Fig. 1 shows the effects of asphyxia, not by vagus stimulation, but by stoppage (twice) of the respiration (which was artificial) in a curarized dog. When the respiration was stopped (at "off"), asphyxia quickly developed. The blood pressure promptly rose. This was due primarily to stimulation of the aortic body by lack of oxygen in the blood (hypoöxia), but also partly to the stimulating action of

carbon dioxide in the venous blood on the vasomotor center in the medulla, and to some further stimulation (by the hypoöxia) of the carotid body. Sensory (chemo-) reflexes pass from the aortic body (over the vagus nerve) and from the carotid body (over the branches of the sinus nerve) to the medullary centers in which the necessary readjustments are made, so that efferent impulses can be sent out over the true sympathetic system to cause the rise in blood pressure. Resumption of respiration (at "on") quickly causes a return to normal as the blood is again reoxygenated.

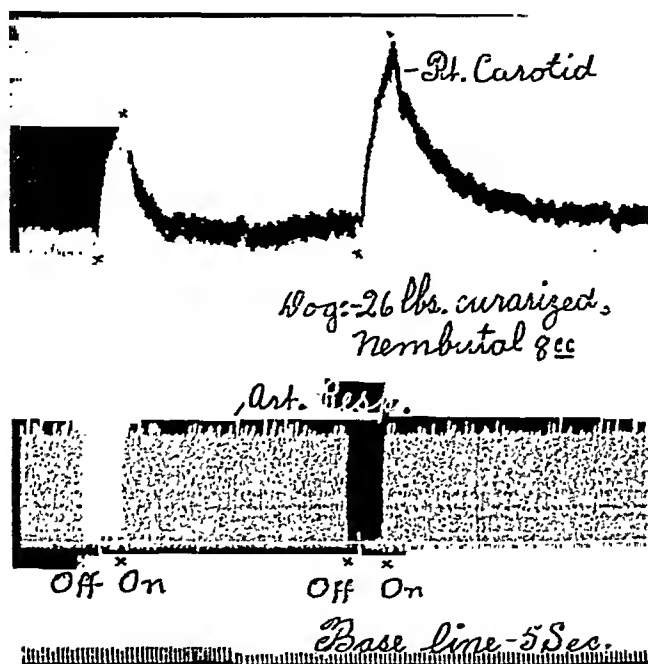


Fig. 1.—For discussion see text.

Fig. 2 shows the action of paredrinol on the walls of the nasal sinuses and passages, on the blood pressure, and on the respiration. Here marked stimulation of the vasoconstrictor nerve endings caused a prompt and prolonged constriction of the vessels in the walls of the nasal passages (nose record) and a rise in blood pressure. As the blood pressure rises, the heartbeat becomes slower (increased pulse pressure shown by the mercury manometer tracing), until finally, at the highest point, the pressure suddenly drops and an extended series of very slow beats ensue. These slow beats are due primarily to the reflex action of the carotid sinus nerves. These nerves (endings) are stimulated by the increased tension (stretch) in the walls of the carotid sinuses, and impulses are quickly sent to the medullary center from which reflex impulses are sent to the heart over the inhibitory vagus fibers. These slow the heartbeat. A vasodilator action (not identifiable in this record) may also have been present but concealed by the peripheral action of the drug. See also discussion of Fig. 12.

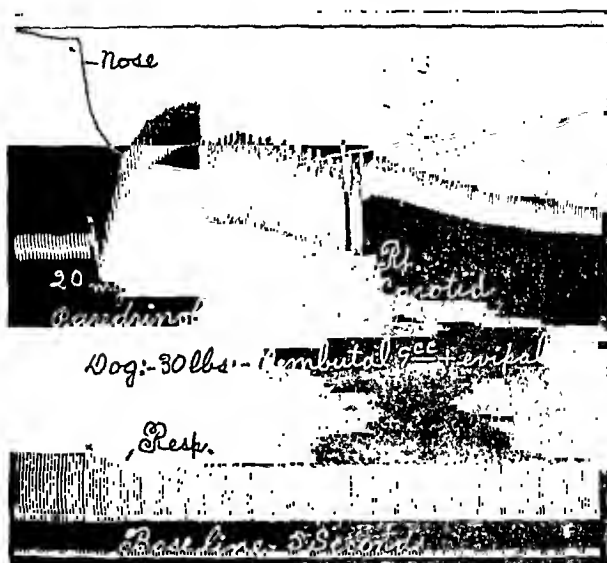


Fig. 2.—For discussion see text. The nose cannula was made by means of a tumbour connected with rubber tubing to one nostril into which a short piece of glass tubing was inserted (airtight). The other nostril was closed tightly by means of a clamp. The soft palate was held firmly pushed back against the posterior wall of the nasopharynx by means of a soft rubber ball attached to the end of a metal rod. The free end of the rod rested tightly against the upper incisor teeth, and the whole rod was held in place by means of a clamp which closed over the rod and around the animal's nose. This arrangement held the posterior nasal cavity closed airtight, so that changes in the vessels of the walls of the sinuses and air passages could be readily recorded by air transmissions to the tumbour.

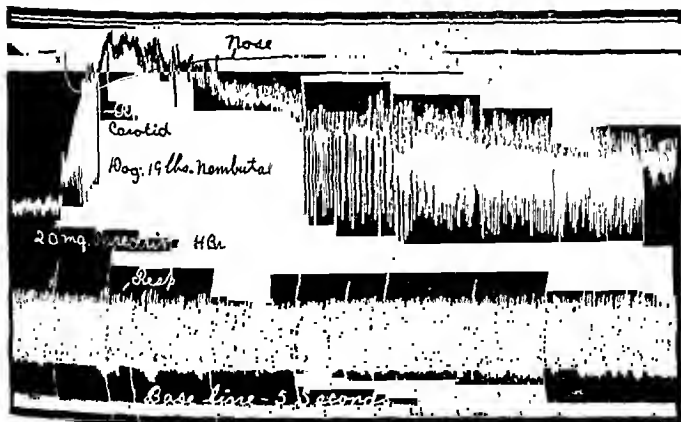


Fig. 3.—For discussion see text

Fig. 3 shows actions similar to those of Fig. 2, but in Fig. 3 paredrine was injected and the inhibitory action of the carotid sinus reflex was very much more extended. Artificial respiration was being carried out in this experiment, and this may have influenced the depth of the anesthesia somewhat. The more lightly the animal is anesthetized, the more active the inhibitory reflexes are likely to be. Formerly this vagus inhibitory action (slow heartbeat) was supposed to be due entirely to mechanical stimulation of the inhibitory center in the medulla by increase in intracranial pressure from the marked vasoconstriction produced (peripherally) by such drugs. Carotid reflexes have been found by various investigators to be greatly reduced, or abolished entirely, by certain anesthetics such as ether and most of the barbiturates. Clinically one might consider whether or not such an action would be beneficial or otherwise to a patient who was anesthetized for an operation.

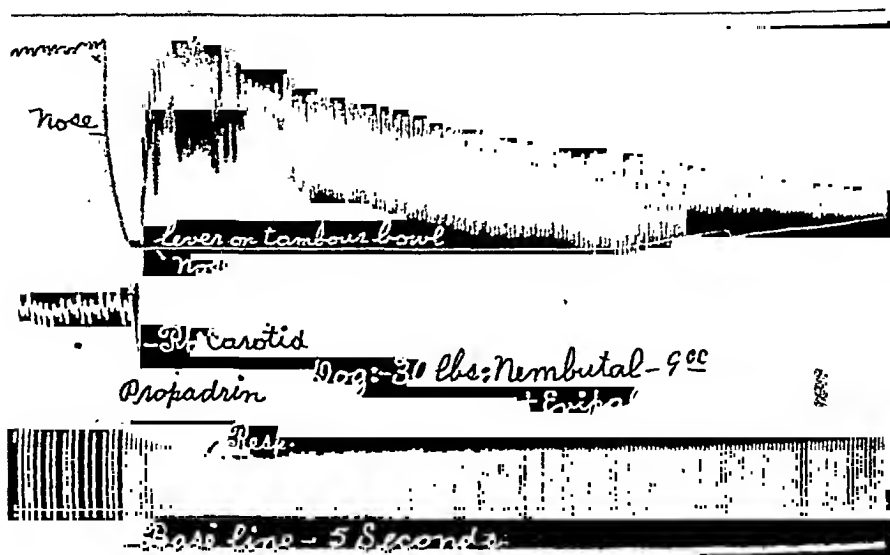


Fig. 4.—For discussion see text.

Fig. 4 illustrates the action of propadrine. A slight stimulation of the respiratory rate is present here, and it is often much more marked, for this and various other members of the sympathomimetic amines often stimulate the central nervous system in addition to their peripheral actions.

Fig. 5 illustrates the action of benzedrine. It should be noted here that the drug was injected into the femoral vein. This is far removed from the mucous membranes of the nasal passages, yet these membranes show an immediate shrinking. The constriction of these vessels is exactly analogous to that which occurs in the kidney or spleen.

Fig. 6 shows the action of neosynephrin, but in this case a striking abnormality in the respiration is noted. This change is a variety of Cheyne-Stokes respiration and is due to the effects of the anesthetic (nembutal) rather than to the neosynephrin. The interrelations between the blood pressure and the respiration should be noted.

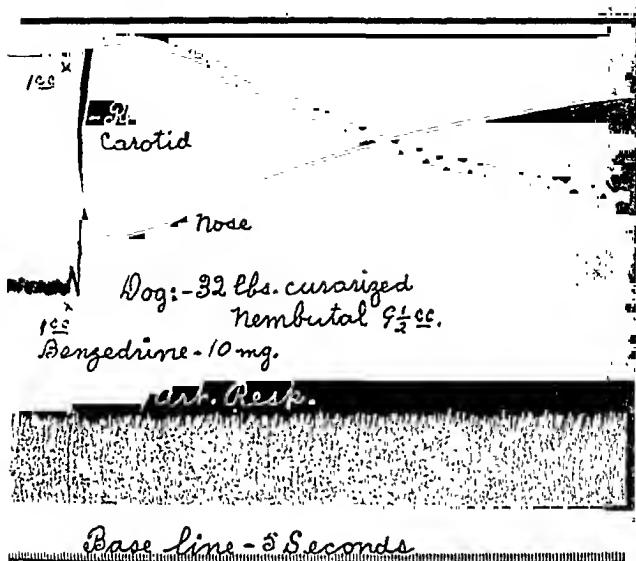


Fig. 5.—For discussion see text.

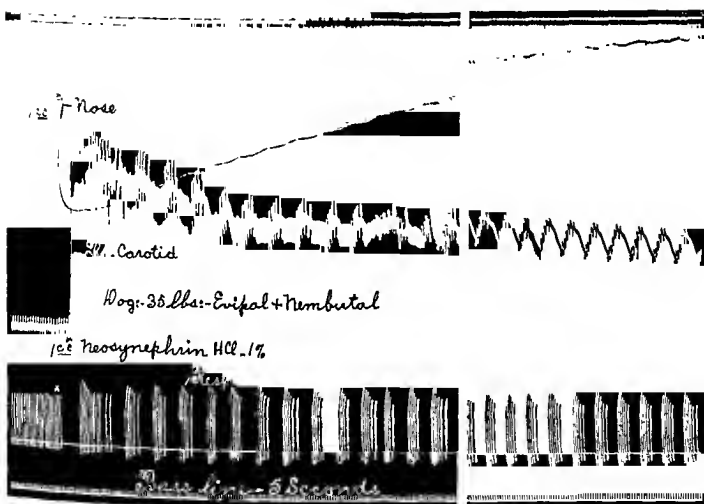


Fig. 6.—For discussion see text. A section of the record was omitted between the two parts here shown.

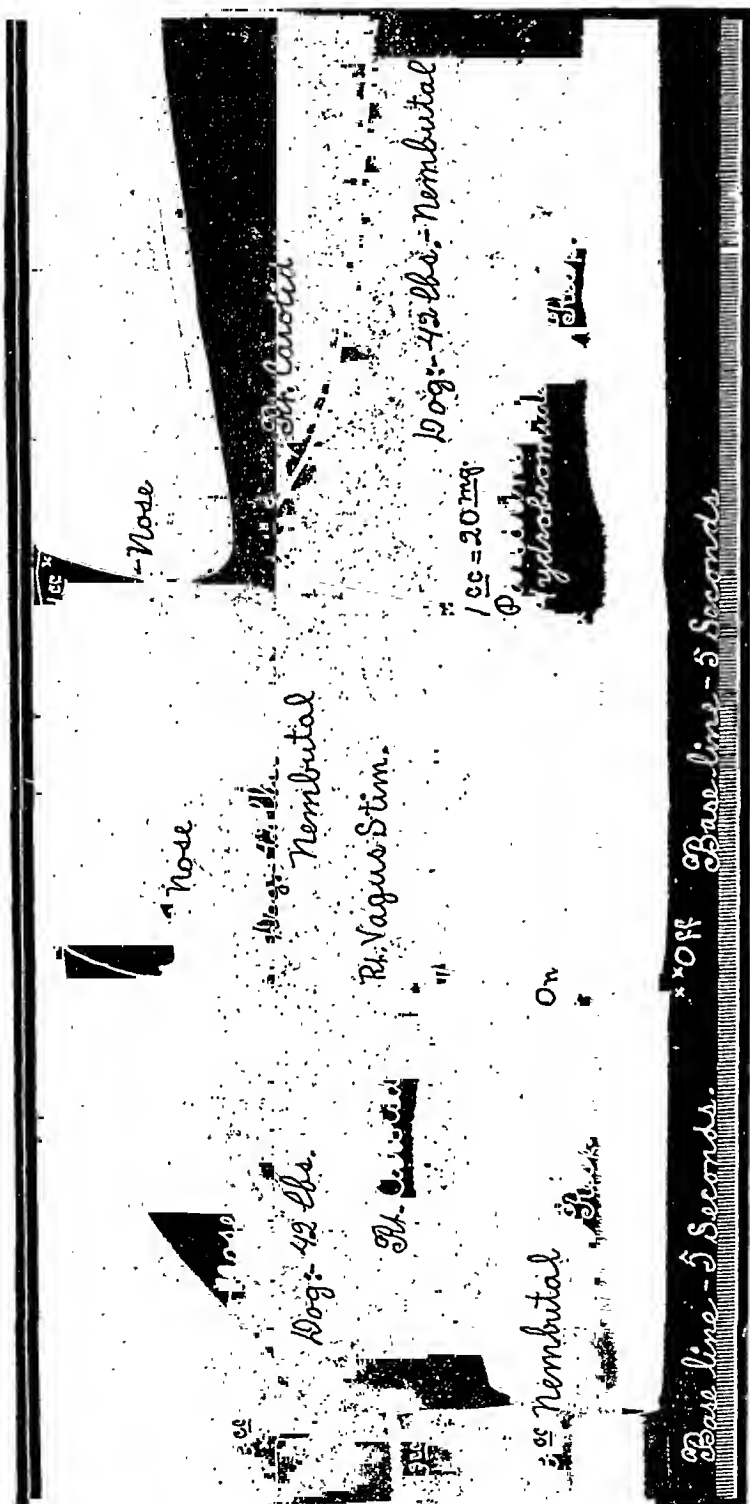


Fig. 7.—Here nembutal dilated the vessels in the nasal walls. Drugs which stimulate the medullary center, or asphyxia, may act in exactly the opposite manner, and cause constriction of these vessels. For discussion see text. Also see discussion of Fig. 11.

Fig. 7 demonstrates a variety of autonomic effects. At the beginning of the tracing injection of 3 c.c. of nembutal solution leads to a marked dilatation of the vessels in the nose. This is perhaps partly peripheral but mainly central in action. It may also be partly due to ganglionic paralysis, for Koppányi¹⁸ has noted that the vagus ganglia in the heart are paralyzed by barbiturates. It is to be noted (near the middle of this record) that stimulation of the right (intact) vagus caused only a slight inhibition of the heart. The sympathetic fibers to the nose (with their synapses in the superior cervical ganglion) were still able to produce a nasal vasoconstriction. Pare-drine still produced its normal reaction. In the cervical region in dogs the sympathetic nerves for the head run (peripherally but up into the head) in the vagus trunks. Hence stimulation of the vagus trunk causes vasoconstriction in the nasal walls.

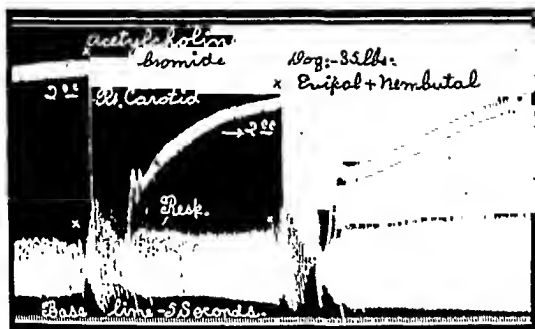


Fig. 8.—For discussion see text.

Figs. 8 and 9 show the action of parasympathetic drugs. The first record shows the effects of two injections of acetylcholine bromide. The rapid recovery from large doses is evident. This is due to rapid destruction of the acetylcholine by esterase at the endings of the vagi nerves in the heart and also in the lungs, for the bronchi are strongly contracted by this drug.

Fig. 9 illustrates the action of arecoline (after nicotine) on the post-ganglionic terminations of the vagi nerves in the heart. This leads to a marked inhibition and weakening of the heart with a corresponding fall in blood pressure, producing an asphyxia which is still further increased by bronchial constriction. The animal resists this asphyxia by increasing and greatly accelerating its respiratory efforts. This results in a somewhat obscuring effect on the respiratory tracing which was made by a tambour connected with a side tube of the tracheal cannula.

Fig. 10 illustrates the ganglionic type of autonomic action. Nicotine first stimulates and then, if the dose is large enough, paralyzes all autonomic ganglia. Acetylcholine (Fig. 8) also has this ganglionic ("nicotine") action, but its peripheral (cholinergic) "muscarine" action may generally over-

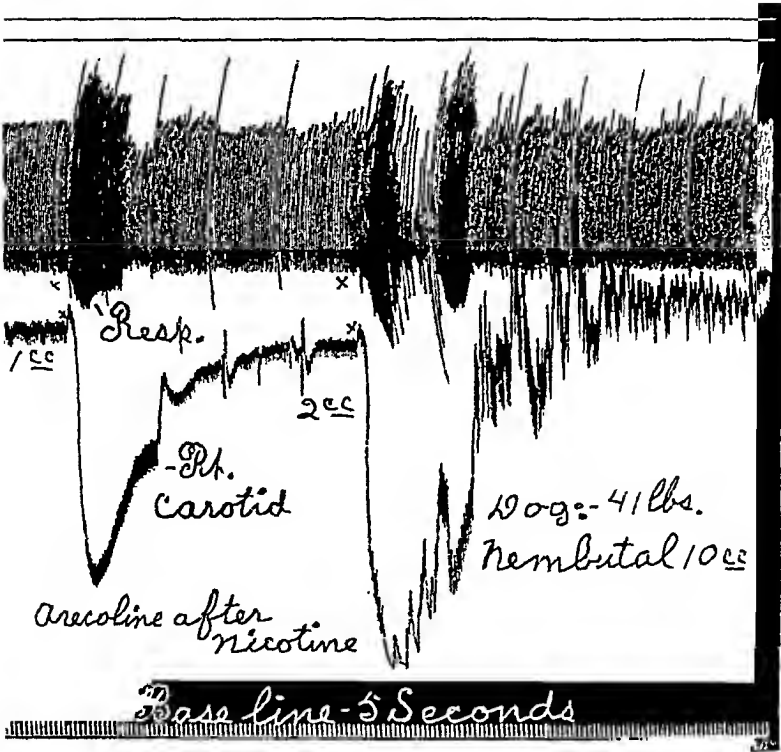


Fig. 9.—For discussion see text.

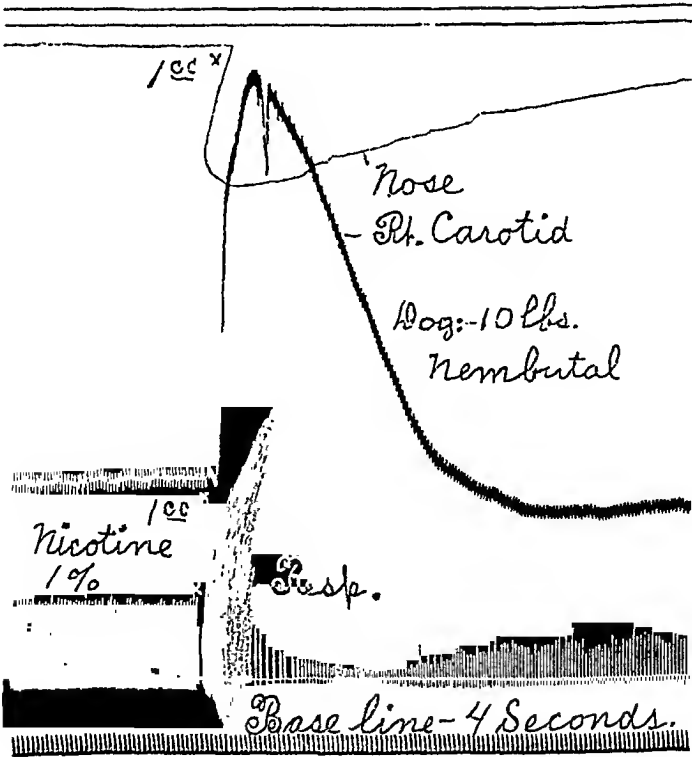


Fig. 10.—For discussion see text.

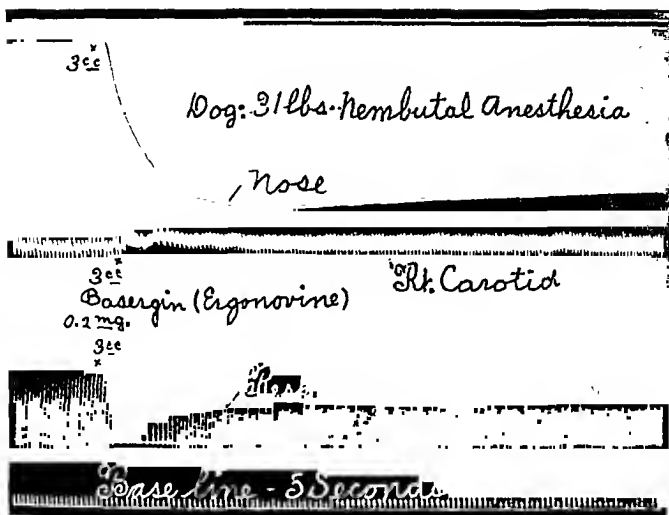


FIG. 11.—For discussion see text.

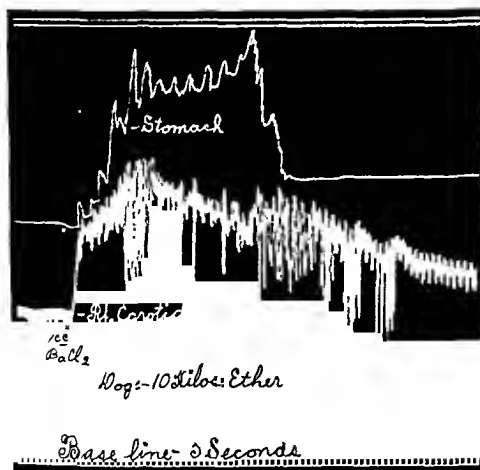


FIG. 12.—For discussion see text. The stomach record was made by a device attached to the pyloric end of the stomach. Contraction is the upward movement.

shadow its ganglionic action. The primary vagus ganglion stimulation is not very evident in Fig. 10, because the dose of nicotine was so large that these ganglia were paralyzed before their inhibitory effects on the heart could be recorded.

In Fig. 11 the action of an ergot alkaloid (basergin, ergonovine) is illustrated. The marked contraction of the vessels of the nose may be largely due to central stimulation as a result of the asphyxia produced by depression of the respiration. This shows the roundabout methods by which the autonomic mechanisms are frequently brought into action. But ergot alkaloids also have a peripheral action resembling that of adrenalin. In this record, however, the slight change in blood pressure rather indicates a different origin for the nasal constriction in which most of the systemic arterioles did not take part.

Fig. 12 demonstrates an entirely different action from any of the other drugs, yet its resemblance to the other records is so close that one must be impressed by the nature of the mechanisms involved. What appear to be very slow beats (carotid sinus inhibition) along the higher parts of the blood pressure tracing are more likely in this instance to be due to extraventricular systoles. This type of action may also occur with the true sympathomimetic amines, and with both types of drugs carotid and aortic sinus reflexes may also occur. With barium, however, the stomach shows marked contraction (muscle stimulation), while with adrenalin-like drugs inhibition generally occurs except at the sphincters (and even these have an obscure innervation).

Barium and some other metals, posterior pituitary extracts (pitressin, pitocin), probably angiotonin, histamine, nitrites, and various other drugs act on smooth muscle to produce either stimulation or depression. These actions are generally considered to be directly on the muscle fibers. Probably the peripheral actions of some anesthetics should be included here. It seems quite possible, and even probable, that some, and maybe all, of these drugs really do effect their actions through nervous elements or receptors which are not yet understood. There are special indications for this view, especially in the case of posterior pituitary extracts.

The nature of the points on which sympathomimetic drugs act has long been discussed. Earlier workers called these points "end plates" or simply "nerve endings." Later the term "myoneural junction" was introduced, and finally the word "receptor." The history of the changing viewpoints which these terms have signaled in the past forty years constitutes one of the most interesting chapters in the records of modern medicine. The very recent introduction of the conception of adrenergic and cholinergic nerve endings paves the way for another, perhaps equally evanescent, chapter.

There are still many obscure phenomena connected with the pharmacology and physiology of the autonomic nervous system; and probably mechanisms of great importance connected therewith have not yet even been surmised. The very recent discovery of the special significances of the carotid and aortic bodies and sinuses, the meanings of which are still far from being completely understood,⁹ serves well to illustrate this. From the standpoint of pathology,

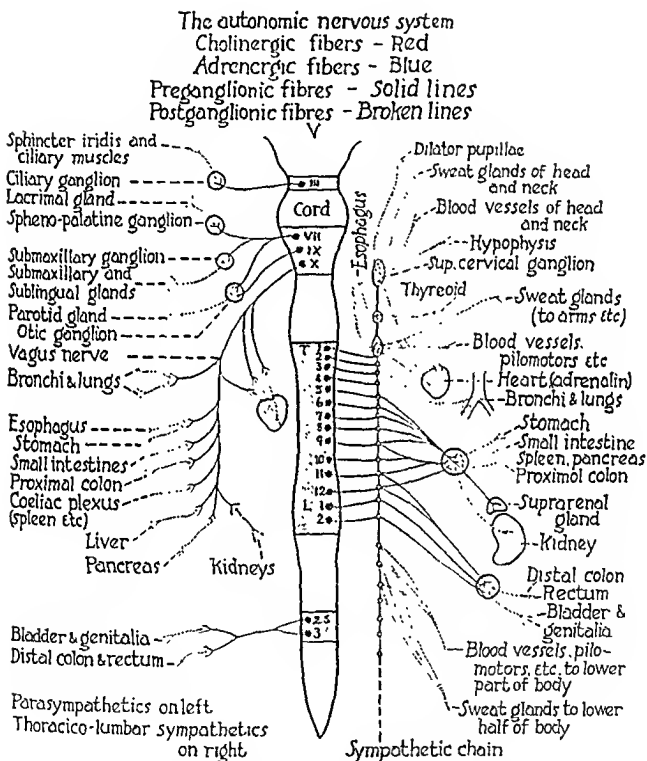


Fig. 13.—Diagrammatic representation of the autonomic nervous system. The parasympathetics are (mostly) shown on the left while the thoracolumbar (true) sympathetics are shown on the right. Note the color scheme to differentiate between adrenergic and cholinergic fibers. (From Jackson: *Experimental Pharmacology and Materia Medica*.) Figs. 1 to 12 illustrate actions at all points at which drugs may affect the autonomic nervous system with the possible exception of some centers in the hypothalamus or other parts of the brain and cord

a mere beginning has been made. Not infrequently one may stand in the presence of the direst clinical phenomena, and feel and see dimly as if in a dream, or sometimes as if in a nightmare, that the symptoms he observes must of necessity be largely, if not completely, due to actions of the autonomic nervous system. And yet as death hovers near, or even seizes the victim, the most astute physician may sometimes not be able to determine what mechanisms are involved, let alone cure the patient. Fatal attacks of angina pectoris, or the origin of the obscure and sometimes fatal attacks in the presence of pheochromocytomata, and probably other causes of death not now recognized clinically, may serve to illustrate this point.

A considerable number of clinical conditions are now known or believed to bear a more or less direct relationship to the autonomic nervous system. Among these may be mentioned vasomotor rhinitis, bronchial asthma, urticaria, angioneurotic edema, colic in hollow visceral organs, motor disturbances of these organs, hiccups, angina pectoris, disturbances of cardiac rhythm, migraine, diabetes insipidus, pyrexia, hypertension, and the like. Undoubtedly a number of others should be added even though their relationship to the autonomic nervous system may not be known until far in the future.

The ancients were accustomed to orient their historical perspective of civilization with reference to their seven wonders of the world. Modern man has added many more wonders. Yet not one of these, either ancient or modern, presents a more remarkable phenomenon than do the actions of some of these drugs on the autonomic nervous system, such, for example, as the action of 1 or 2 mg. of atropine or adrenalin when injected intravenously into a man weighing as much as 150 pounds.

REFERENCES

1. Gaddum, J. H., and Kwiatkowski, H.: Inactivation of Adrenalin by Amine Oxidase, *J. Physiol.* 94: 87, 1938.
2. Richter, Derek: The Inactivation of Adrenalin in Vivo in Man, *J. Physiol.* 98: 361, 1940.
3. Page, Irvine H.: Difference in the Activating Effect of Normal and Hypertensive Plasma on Intestinal Segments Treated With Renin, *Am. J. Physiol.* 130: 29, 1940.
4. Prinzmetal, Myron, Lewis, Harvey, and Leo, Sydney: Etiology of Hypertension Due to Complete Renal Ischemia, *Proc. Soc. Exper. Biol. & Med.* 43: 694, 1940.
5. Dock, William: Vasoconstriction in Renal Hypertension Abolished by Pituitary, *Am. J. Physiol.* 130: 7, 1940.
6. Marrazzi, Amadeo S.: Adrenergic Inhibition at Sympathetic Synapses, *Am. J. Physiol.* 127: 738, 1939.
7. Herwick, Robert P., Linegar, Charles R., and Koppanyi, Theodore: The Effect of Anesthesia on the Vasomotor Reversal, *J. Pharmacol. & Exper. Therap.* 65: 185, 1939.
8. Linegar, Charles R., Dille, James M., and Koppanyi, Theodore: Analysis of a Peripheral Action of Barbiturates, *J. Pharmacol. & Exper. Therap.* 58: 128, 1936.
9. Gesell, Robert, Lapiques, Jack, and Levin, Manuel: The Interaction of Central and Peripheral Control (Respiration), *Am. J. Physiol.* 130: 155, 1940.

INTERACTION OF VITAMINS AND DRUGS WITH CELL CATALYSTS*

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TO THE clinician and physiologist the results obtained with isolated enzymes and tissue suspensions may seem to have only remote application to the processes in the human and intact animal. It is the purpose of this review to correlate, in certain selected instances, the physiologic and pathologic findings in man and animals with what is known of the concomitant changes in tissue metabolism and the catalysts involved.

In the first part, four vitamins will be considered. Each of these vitamins, or its derivative formed in the body, combines with a specific protein and this combination is an enzyme, the absence of which in deficiency states causes metabolic disturbances which may partly account for the accompanying symptoms. In the second part, drugs will be discussed which either (a) combine with specific enzymes, thereby inhibiting their normal catalytic activity, or (b) are attacked and rendered pharmacologically inactive by enzymes.

PART I

Thiamin: (a) Carbohydrate Metabolism.—The work on thiamin may be divided into two parts. The first definite evidence about the nature of the disturbance in the central nervous system in thiamin deficiency was obtained by Peters.¹ He showed that the minced optic lobes of the pigeon with beriberi were unable to oxidize pyruvic acid and that the addition of small amounts of thiamin in vitro restored the oxidation. Lohmann and Schuster² identified the substance necessary for the decarboxylation of pyruvic acid by yeast as thiamin pyrophosphate. This is also the active form in the animal body,³ which combines with a specific protein to form the enzyme which oxidizes pyruvic acid to acetic acid.

It follows from this that pyruvic acid should accumulate in the blood of thiamin-deficient animals. This has been shown for pigeons⁴ and man,^{5,6} although in the latter other keto acids beside pyruvic are also present. Feeding thiamin reduces the keto acid level of the blood to normal. That pyruvic acid is a normal intermediate in carbohydrate metabolism in man was shown by Johnson and Edwards,⁷ who found high values in the blood after severe exercise. The impairment of this important phase of carbohydrate metabolism in thiamin deficiency probably accounts for the decreased glucose tolerance.⁸ The relation of thiamin to insulin and diabetes is still not clear.

It is not surprising to find a close relationship between thiamin and the thyroid because of the marked effect of this gland on carbohydrate metabolism. There are no in vitro experiments to explain the exact mechanism of the interaction, but the experiments on animals show clearly that thiamin counteracts the toxic action of thyroxine,⁹ prevents weight loss¹⁰ and loss of liver glycogen¹¹

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in thyroid-fed rats, and is helpful in the preoperative treatment of Graves' disease.¹² Conversely, thyroid feeding increases the thiamin requirement of dogs¹³ and depletes the thiamin in the kidney and liver of rats.¹⁴

(b) *Interaction with Acetylcholine.*—The central nervous symptoms of beriberi can be explained by the disturbance in pyruvic acid metabolism. The edema can also be explained on this basis because the pyruvic acid metabolism of the kidney, and consequently kidney function in general, is very sensitive to thiamin deficiency.¹⁵ It is difficult, however, to explain on this basis the loss of appetite, achlorhydria, loss of tone in the gastrointestinal tract and arterioles, and tachycardia, especially as pyruvic acid in concentrations found in beriberi has no toxic action.¹⁶ The following experiments with thiamin and acetylcholine offer a possible explanation for these symptoms. (It is not the purpose of this review to enter into the debate on the exact function of acetylcholine in nervous transmission. It is generally agreed, however, that acetylcholine is produced at most synapses and myoneural junctions, and it follows that a disturbance in acetylcholine metabolism will be reflected in the tissues supplied by cholinergic nerves. The enzyme cholinesterase specifically hydrolyzes acetylcholine.)

Thiamin augments the action of acetylcholine on the blood pressure of the cat and on the isolated rat intestine.¹⁷ It sensitizes various nerve trunks to electrical stimulation,¹⁸ and it is liberated when cholinergic nerves are stimulated electrically.¹⁹ Most of these effects could be explained on the basis of cholinesterase inhibition by thiamin. Thiamin does inhibit the cholinesterase,²⁰ although a relatively large concentration is required. There exists the possibility that, because of physical conditions or local concentration effects, thiamin, *in vivo*, does control cholinesterase activity. Its affinity for the enzyme is twenty-six times that of acetylcholine. But whatever the mechanism of the interaction between thiamin and acetylcholine, such interaction can explain the cardiac and gastrointestinal symptoms of thiamin deficiency, symptoms which can be relieved temporarily by choline esters. Also part of the protective effect of thiamin in hyperthyroidism may be due to its inhibiting action on the serum cholinesterase, which is higher than normal in patients with this disease.²¹

Riboflavin.—Warburg and Christian¹ first demonstrated the catalytic importance of riboflavin in yeast. As a phosphate ester² attached to a specific protein, it forms an enzyme which oxidizes cozymase (see under nicotinic acid) which in combination with another protein is reduced by hexose phosphate. Its importance in animal metabolism was shown when riboflavin was identified as vitamin G.³ Recent *in vitro* work on various oxidases of animal origin have shown that dinucleotide derivatives of riboflavin, rather than riboflavin itself, form important enzymes when combined with specific proteins. These dinucleotide derivatives are decreased in the heart and liver (but not in the brain and kidney) of rats on flavin-deficient diets and are rapidly synthesized when pure riboflavin is fed.⁴ The following enzymes, which are found in a variety of tissues, contain a flavin dinucleotide: Diaphorase 1 and 2 which oxidize reduced cozymase (coenzyme I) and coenzyme II^{5, 6} and which are in turn oxidized through an intermediary by the cytochrome-cytochrome oxidase system; xanthine oxidase, which oxidizes xanthine and hypoxanthine to uric acid

and oxidizes aldehydes to acids;⁷ and d-amino acid oxidase which oxidizes the "nonnatural" isomers of the amino acids to keto acids.⁸ Other less well-defined enzymes have also been described.

It follows that the activity of these enzymes should be diminished in flavin-deficient animals. This has been shown in the rat for xanthine oxidase and d-amino acid oxidase.⁹ The over-all oxygen uptake of tissues taken from flavin-deficient rats is, however, little affected, with the exception of the diaphragm which is increased 30 per cent¹⁰ and the skin which is decreased 37 per cent.¹¹ This latter finding may account for the characteristic alopecia and dermatitis in rats and the cheilosis and seborrheic lesions in man.^{12, 13} The general weakness, fall in body temperature, sudden collapse in deficient dogs,^{14, 15} and lack of growth in rats and chicks¹⁶ can conceivably be explained by the impairment of certain essential oxidases. It is more difficult to explain the cataract¹⁷ and corneal vascularization¹⁸ in rats, the intestinal hemorrhage,¹⁵ and degeneration of the axis cylinders in the posterior columns in dogs¹⁹ on this basis. These symptoms may be the indirect expression of a general metabolic disturbance. There is also no explanation for the effect of riboflavin on the regeneration of hemoglobin in the anemic dog.²⁰

Nicotinic Acid.—Warburg and co-workers¹ first demonstrated the biologic importance of nicotinic acid when they showed that its amide was part of a compound containing as well two pentose, one adenine, and three phosphate molecules. They named this substance coenzyme II and showed that, in combination with a specific protein, it formed an enzyme which oxidized a hexose phosphate. The sugar was oxidized by reducing the coenzyme which in turn was reoxidized by an enzyme containing riboflavin (see under riboflavin). Nicotinic acid amide was then shown to be a part of coenzyme I^{2, 3} which differed from coenzyme II in having one less phosphate molecule and which was identical with cozymase, the substance that Harden and Young⁴ many years previously had shown to be essential for the fermentation of yeast. One or the other of the coenzymes has been found to be essential for the oxidation of important metabolites, for example, lactic acid⁵ which requires coenzyme I. (The oxidation of lactic acid thus needs a nicotinic acid derivative, which in turn needs a riboflavin derivative, and the pyruvic acid formed from the lactic acid then requires thiamin for its oxidation.)

When nicotinic acid was found to be the specific cure for black tongue in dogs⁶ and for pellagra in human beings,⁷⁻⁹ the possibility arose that some of the symptoms might be explained on the basis of a low coenzyme concentration in the tissues, with a consequent impairment of metabolic processes. Since no chemical methods were available for the estimation of the normally very small amounts of coenzymes present in tissues, Kohn¹⁰ devised a biologic test based on the fact that *H. parainfluenzae* required coenzyme I and/or II for growth.¹¹ Although this test seems specific for the two coenzymes, there remains the possibility that other similar, but as yet unknown, compounds may act as growth factors for these bacteria. With this method, the normal coenzyme value of human blood was determined, and it was shown that all the coenzyme was carried in the corpuscles. The value for pellagrins was within the normal range. This was confirmed later in a larger series of cases,¹² and Axelrod and asso-

ciates^{12a} obtained similar results. The low values obtained by Vilter, Vilter, and Spies¹³ for pellagrins might be explained by their use of different bacteria, or by the fact that their results were expressed in terms of whole blood instead of corpuscles. Kohn was also able to show that the oral administration of nicotinic acid rapidly raises the coenzyme concentration in the red blood cells of normal subjects and pellagrins.

Further investigation of this finding¹⁴ showed that human corpuscles were able to synthesize coenzyme from nicotinic acid *in vitro* and that they were then able to oxidize lactic acid more rapidly. The inability of Vilter, Vilter, and Spies¹⁵ to obtain this synthesis of coenzyme from nicotinic acid with washed cells is explained¹⁶ by the fact that their method of washing definitely injures the cells.

The fact that the blood of human pellagrins showed no marked variation from normal in coenzyme concentration made it necessary to study the concentration in the tissues. For this the black tongue dog was used.¹⁷ It was found that the coenzyme of the liver was reduced 70 per cent and that of striated muscle 35 per cent in black tongue, but that other tissues had normal values. These results, which were confirmed by Axelrod and associates,¹⁸ indicate that the liver was sacrificing its store of coenzyme to maintain the level in other tissues. The oxygen uptake of the isolated liver tissue was, however, increased 35 per cent (see oxygen uptake of diaphragm in riboflavin deficiency).

No symptom of black tongue or pellagra can be directly explained on the basis of impaired metabolism caused by deficiency in coenzyme. It is always possible that the symptoms are an indirect expression of such an impairment, but the *in vitro* results leave open the possibility that nicotinic acid may have other functions in the body.

The concentration of coenzyme in the blood has been measured in a variety of diseases. It tends to be low in diabetes^{12, 19} and high in pneumonia,¹² but it is normal in cardiovascular and gastrointestinal disturbances.¹² It is very low in tumor and embryonic tissue.^{20, 21}

Vitamin A.—Of the various actions of vitamin A in the animal, the only one that has been elucidated by *in vitro* experimentation is its action in the eye. The relationship of diet to night blindness was recognized by a number of investigators, but was clearly stated by Bloch.¹ Fridericia and Holm² and Holm³ were able to show that the regeneration of visual purple after bleaching with light took place more slowly in vitamin A-deficient rats which were night blind. The normal pig eye was then found to be one of the richest sources of vitamin A.⁴ The correlation between a low concentration of vitamin A in the blood and night blindness in human subjects was made by Sic.⁵ Numerous dark adaptation tests have been made^{6, 7} in an attempt to use this as a measure of vitamin A deficiency, and it has been shown⁸ that administration of vitamin A to human subjects deficient in vitamin A causes rapid improvement in their dark adaptation.

The exact part that vitamin A plays in rod vision and its relation to rhodopsin (visual purple) was first elucidated by Wald.^{9, 10} He was able to show that the following cyclic mechanism occurs. Illumination rapidly bleaches rhodopsin even in solution extracted and separated from the retina, and the yellow

pigment retinene is formed. Some of the retinene can be directly reconverted in the dark into rhodopsin, but some is converted into vitamin A. These two reactions occur to a slight extent in solution but take place readily in the isolated retina. The vitamin A formed is then slowly reconverted into rhodopsin. This reaction, however, occurs only in the intact eye, where rhodopsin, retinene, and vitamin A are all loosely combined with protein.

This mechanism explains not only the night blindness in A deficiency, but also the rapid dark adaptation following an intense flash of light on the dark adapted eye. During the short flash, a large amount of retinene accumulates, but relatively little is converted to vitamin A. In the dark, retinene is rapidly converted into rhodopsin. After long illumination a steady state is reached with a small amount of retinene and a relatively large amount of vitamin A which is now the main source of the rhodopsin. Since this conversion is relatively slow, dark adaptation after prolonged illumination should be slower than after the short flash, and experimentally this is found to be so. According to the Janesos,¹¹ the pigmented epithelium converts vitamin A into rhodopsin which then migrates into the rods, and retinene migrates from the rods to the pigment cells where it is converted into vitamin A.

Wald¹² was also able to show that mammals and salt-water fish use vitamin A₁, whereas fresh-water fish use vitamin A₂ in their visual cycle. The differences in the absorption bands of A₁ and A₂ are carried over when they are converted into rhodopsin and porphyropsin, respectively, and into retinene 1 and 2. From the chick eye, which is almost completely made up of cones, Wald¹³ has extracted another pigment, iodopsin, which is also a protein-carotenoid complex. It seems probable that vitamin A plays some part in the cones of the human eye, as several investigators^{8, 14, 15} have found that cone and rod thresholds vary in the same way in vitamin A deficiency.

PART II

Eserine (Physostigmine) and Prostigmine.—Dale,¹ Brown,² and Cannon³ have reviewed the evidence that the production of acetylcholine is part of the mechanism for synaptic and myoneural transmission. The cholinesterase, which hydrolyzes acetylcholine into choline and acetic acid, thus making it inactive, is an intimate part of this mechanism. It follows that any drug that inhibits the activity of this specific enzyme and thus allows acetylcholine to accumulate in vitro may have definite effects when injected into the animal. In assessing how far the in vitro effects are paralleled by the in vivo action of such drugs, the distribution of the drug in the body and the consequent inhibition of the esterase in situ must be taken into consideration. For instance, two drugs may inhibit a solution of esterase to the same extent, but when injected into the animal, one may cause excitation, the other depression (see below), either because their locus of action is different or because different amounts of acetylcholine are allowed to accumulate. In most ganglia, acetylcholine in small amounts causes excitation; in larger amounts, depression.^{4, 5}

Kahane and L  vy,⁶ using the single drug, eserine, showed that the effects of various choline esters in the body were potentiated by eserine in proportion to the rapidity with which the different esters were hydrolyzed by the cholinesterase.

terase. But when several drugs and one ester were used, there was no correlation between the extent of the inhibition *in vitro* and the potentiating effect *in vivo*,⁷ presumably because the drugs varied in their ability to penetrate to the enzyme *in situ*. Such considerations may also explain the results of Schweitzer and Wright⁸ who showed that eserine increases reflex excitability in the cat and that prostigmine depresses it.

Despite this and other minor variations, the effects of eserine and prostigmine injected into the animal closely parallel the effects of acetylcholine, and in all cases potentiate the effects of the latter. This has been shown for the human eye,⁹ respiration in cats,¹⁰ sympathetic ganglia,⁷ mammalian skeletal muscle,¹¹ the human gastrointestinal tract,¹² when injected into the ventricles of man,¹³ and on the T-wave in the dog heart.¹⁴ Clark and Raventos¹⁵ have shown that the action of eserine on the frog heart can be accounted for quantitatively by its inhibition of the esterase. Eserine and prostigmine, when injected, decrease the esterase activity of the circulating blood as has been demonstrated many times in myasthenia gravis. (For the literature on the treatment of this disease by prostigmine, see reference 16. The symptoms of myotonia are increased by injection of prostigmine¹⁷).

Morphine.—The effect of various centrally acting drugs on the cholinesterase of the brain has been investigated.¹⁸ Morphine and apomorphine were found to cause significant inhibitions in low concentrations. Because intraventricular injections of acetylcholine in man¹¹ and injections into the hypothalamic regions in cats¹⁹ usually cause nausea and vomiting, it is possible that morphine and apomorphine may produce these effects through the accumulation of acetylcholine in regions affecting the vomiting center. Kuhn and Surles²⁰ investigated other compounds of the morphine group and found a parallelism between the central emetic action and the inhibitory effect on the esterase *in vitro*. Moreover, emetine was the only other drug tested that inhibited the enzyme in equivalent concentrations. The blood and tissue cholinesterase was inhibited in the same way by these drugs. Besides giving a possible explanation for the central emetic action, this inhibition by morphine could also explain its other effects in the body, such as the slowing of the heart, increase of spinal reflexes, and spasticity of the intestine with constipation. Recently, Slaughter and Gross²¹ and Slaughter and Mimsell²² have confirmed these findings and have shown that the inhibition occurs *in vivo*. They have also shown that eserine potentiates the action of morphine and that with small doses of eserine it is possible to decrease the therapeutic dose of morphine by half. This suggests that sensory impulses may also be mediated by acetylcholine. The fact that strychnine²³ also inhibits the cholinesterase may explain how it increases the spinal reflexes. Its other effects which differ from those of morphine may well be explained on the different distribution of these two drugs in the body.

Amines (Except Histamine), Adrenaline, and Sympathomimetic Substances. An enzyme which specifically oxidizes certain amines was first isolated from the liver and studied by Hare¹ and Bernheim.² It was thought that this enzyme had a protective function in the liver in oxidizing amines formed by bacterial decarboxylation of amino acids in the intestine. The aldehydes formed from this oxidation which were no longer pharmacologically active could re-

enter into normal metabolic processes. The fact that animals, in which the cecum is large, as in the guinea pig and rabbit, have large concentrations of this enzyme in their livers supports this idea, as does the fact that mesecaline, the active principle of the Mexican cactus (*Anhalonium lewinii*), is also oxidized by the enzyme.³ In this case an acid is formed which is identical with that isolated from the urine of men fed mesecaline.⁴ The finding of amine oxidase in the brain⁵ and in a number of other tissues,⁶ and the identification of the adrenaline-oxidizing catalyst⁷ with it⁸ suggest other functions for this enzyme.

Because of the two hydroxy groups in the ortho position in the benzene ring, adrenaline is an autooxidizable compound which in oxygen is oxidized to a melanin-like substance. It was thought that this oxidation accounted for the evanescent effects of injected adrenaline. It was shown, however, that body fluids, such as blood and lymph,⁹ extracts of heart,⁹ extracts of uterus,¹⁰ and mixtures of amino acids,¹¹ especially cysteine and glutathione,¹² delay or prevent this oxidation. The amine oxidase, therefore, may be important for the oxidation of adrenaline, although its affinity for the enzyme is low.¹³ The products of the oxidation are ethylamine and an aldehyde which is inactive.¹⁴ The two hydroxy groups are not oxidized by the enzyme. It has been suggested¹⁵ that the amine oxidase may oxidize the adrenaline-like substances (sympathin) produced at certain postganglionic sympathetic nerve endings, although its presence at such endings has not yet been demonstrated. Ephedrine and benzedrine inhibit the amine oxidase,¹⁶ and it is possible to explain their peripheral action on this basis, i.e., the prolongation of sympathetic effects.

The amount of central excitation produced by benzedrine, ephedrine, and allied substances closely parallels the amount of inhibition produced by them on the amine oxidase in vitro.¹⁴ This raises the question of the function of the amine oxidase in the brain. Mann and Quastel¹⁷ have shown that aldehydes formed from the oxidation of amines such as tyramine markedly inhibit the respiration of the brain. Benzedrine, by inhibiting the amine oxidase, prevents the production of such aldehydes and thus increases the brain respiration and may thereby produce excitation. This explanation assumes that small concentrations of aldehyde regulate directly or indirectly central excitation, and conversely that an increase in the amount of aldehyde may lead to narcolepsy and similar depressed states.

An attempt has been made to show that the potentiating action of cocaine and related substances on the sympathetic system is due to the inhibition of the amine oxidase by these drugs.¹⁸ Inhibition has been found to occur, but only in the presence of exceedingly high concentrations of these drugs. It is possible that the enzyme in situ is more sensitive.

Other mechanisms for the oxidation of adrenaline have been suggested. It is oxidized to form a red pigment, adrenochrome, by certain dehydrogenases, and the pigment in turn is oxidized by the cytochrome system.¹⁹ This may be the mechanism of the "pyrocatechol oxidase" investigated by Heirman²⁰ and Baer²¹ which oxidizes adrenaline to adrenoxine, a compound that lowers the blood pressure of rabbits and slows the frog heart.²¹

Histamine.—Although the original finding of Barger and Dale¹ of histamine in the intestinal mucosa raised the question of whether it was actually present

in the cell or formed by the methods of extraction, it is now recognized as a normal constituent of most cells.² In the dog³ the largest concentration of histamine is in the duodenum, lung, and liver, respectively. Within the cell it apparently has no pharmacologic action, but when released into the blood it gives many symptoms of shock. With modern micromethods for its estimation, the concentration of histamine in the blood in various conditions has been measured. Blood under normal conditions contains very small amounts. It is increased in perfusates from the lungs of sensitized guinea pigs⁴ and from the lungs of guinea pigs perfused with snake venom,^{5,6} staphylococcus toxin,⁷ and peptone.⁸ It is increased in the blood of dogs and guinea pigs during anaphylactic shock^{9,10} and insulin shock¹¹ and is released from tissues injured by various chemical and physical agents.¹² Its concentration is high in the blood of patients with migraine, asthma, urticaria, and other allergic diseases,¹³ and when injected, it increases migraine headache, probably by increasing the pulsations of the pial and dural arteries.^{14,15} Histamine will cause spontaneous contractions in the isolated auricle of the guinea pig¹⁶ and is produced continuously by the dog heart,¹⁷ the amount produced being a function of the work done by the heart.¹⁸ Its production by the normal heart, however, has been questioned.¹⁹ It is also produced by active skeletal muscle,²⁰ by sensitized red blood cells *in vitro*,²¹ and in the skin by painful stimuli.²²

The presence of an enzyme in tissues that inactivates histamine was first described by Best and McHenry²³ and studied by McHenry and Gavin.²⁴ Recently it has been studied in detail,^{25,26} and it has been shown to produce ammonia and hydrogen peroxide and to require oxygen for its action on histamine. It also oxidizes cadaverine and putrescine,²⁷ and the name *diaminoxidase* has been proposed for it instead of the original *histaminase*. The enzyme is rapidly inactivated by acid and by trypsin and pepsin.^{26,28} It is present in the tissues of most animals, and in man the kidney has more than the liver.²⁹

Preparations of the enzyme given twelve hours before the antigen to sensitized guinea pigs decreases the severity of the anaphylactic shock,³⁰ and it has been claimed that histaminase in the blood of allergic patients is lower than normal.³¹ A commercial preparation from the intestinal mucosa called *torantil* has been used for various allergic conditions, especially serum sickness^{32,33} and hypersensitivity to cold.^{34,35} It is given in salol-coated pills to prevent its destruction by acid in the stomach, and on liberation in the intestine it must be rapidly absorbed into the mucosa to prevent its destruction by trypsin. In the mucosa it may lower the concentration of histamine by oxidative deamination, and thus lower the concentration in the blood, which presumably is higher than normal in allergic cases. The best preparations of the enzyme act very slowly *in vitro*, requiring twenty-four hours at 37° C. to oxidize 1.0 mg. of histamine. Any rapid improvement cannot be expected from the administration of the enzyme, and further work is required before its clinical value can be definitely established. Equivocal results with the enzyme have recently been obtained.³⁶

Because of lack of space, no discussion is possible of the enzymes in tissues that hydrolyze atropine and homatropine³⁷ and acetanilid,³⁸ or of the effect of various anesthetics on the enzyme systems of the brain.³⁹

REFERENCES

Thiamin:

1. Peters, R. A.: *Biochem. J.* 30: 2206, 1936.
2. Lohmann, K., and Schuster, P.: *Biochem. Ztschr.* 244: 188, 1937.
3. Stern, K. G., and Melnick, J. L.: *J. Biol. Chem.* 131: 597, 1939.
4. Thompson, R. H. S., and Johnson, R. E.: *Biochem. J.* 29: 694, 1935.
5. Platt, B. S., and Lu, G. D.: *Quart. J. Med.* 5: 355, 1936.
6. Shindo, T.: *Ztschr. f. physiol. Chem.* 247: 111, 1937.
7. Johnson, R. E., and Edwards, H. T.: *J. Biol. Chem.* 118: 427, 1937.
8. Chureh, C. P., and Whipple, D. V.: *Am. J. M. Sc.* 193: 733, 1937.
9. Sure, B., and Buchanan, B. S.: *J. Nutrition* 13: 513, 1937.
10. Peters, R. A., and Rossiter, R. J.: *Biochem. J.* 33: 1140, 1939.
11. Drill, V. A.: *J. Nutrition* 14: 355, 1937.
12. Frazier, W. D., and Ravdin, I. D.: *Surgery* 4: 680, 1938.
13. Himwies, H. E., Goldfarb, W., and Cowgill, G. R.: *Proc. Soc. Exper. Biol. & Med.* 28: 646, 1931.
14. Drill, V. A.: *Am. J. Physiol.* 122: 486, 1938.
15. Peters, R. A., and Thompson, R. H. S.: *Biochem. J.* 28: 916, 1934.
16. deJong, S.: *Arch. néerl. de physiol.* 21: 465, 1936.
17. Agid, R., Beauvallet, M., and Minz, B.: *Compt. rend. Soc. de biol.* 126: 982, 1937.
18. Minz, B., and Agid, R.: *Compt. rend. Soc. de biol.* 205: 576, 1937.
19. Minz, B.: *Compt. rend. Soc. de biol.* 127: 1251, 1938.
20. Gliok, D.: *J. Pharmacol.* 65: 389, 1939.
21. Antopol, W., Tuchman, I., and Schiffrin, A.: *Proc. Soc. Exper. Biol. & Med.* 36: 46, 1937.

Riboflavin:

1. Warburg, O., and Christian, W.: *Biochem. Ztschr.* 254: 438, 1932.
2. Theorell, H.: *Biochem. Ztschr.* 275: 37, 1934.
3. György, P., Kuhn, R., and Wagner-Jauregg, T.: *Naturwissensch.* 21: 560, 1933.
4. Oehoa, S., and Rossiter, R. J.: *Biochem. J.* 33: 2008, 1939.
5. Euler, H. v., and Hellström, *Ztschr. f. physiol. Chem.* 252: 31, 1938.
6. Abraham, E. P., and Adler, E.: *Biochem. J.* 34: 119, 1940.
7. Ball, E. G.: *J. Biol. Chem.* 128: 51, 1939.
8. Warburg, O., and Christian, W.: *Biochem. Ztschr.* 298: 150, 1938.
9. Axelrod, A. E., Sober, H. A., and Elvehjem, C. A.: *Nature* 144: 670, 1939.
10. Hastings, A. B., Muus, J., and Bessey, O. A.: *J. Biol. Chem.* 129: 295, 1939.
11. Adams, P. D.: *J. Biol. Chem.* 116: 641, 1936.
12. Sebrell, W. H., and Butler, R. E.: *Pub. Health Rep.* 53: 2282, 1938.
13. Jolliffe, N., Fein, H. D., and Rosenblum, L. A.: *New England J. Med.* 221: 921, 1939.
14. Sebrell, W. H., and Onstott, R. H.: *Pub. Health Rep.* 53: 83, 1938.
15. Axelrod, A. E., Lipton, M. A., and Elvehjem, C. A.: *Am. J. Physiol.* 128: 703, 1940.
16. Lepkovsky, S., and Jukes, T.: *J. Biol. Chem.* 111: 119, 1935.
17. Day, P. L., Darby, W. J., and Cosgrove, K. W.: *J. Nutrition* 15: 83, 1938.
18. Bessey, O. A., and Wolbaeh, S. B.: *J. Exper. Med.* 69: 1, 1939.
19. Zimmerman, H. M., Cowgill, G. R., and Fox, J. C.: *Arch. Neurol. & Psychiat.* 37: 286, 1937.
20. György, P., Robschey-Robbins, F. S., and Whipple, G. H.: *Am. J. Physiol.* 122: 154, 1938.

Nicotinic Acid:

1. Warburg, O., Christian, W., and Griese, H.: *Biochem. Ztschr.* 282: 157, 1935.
2. Warburg, O., and Christian, W.: *Biochem. Ztschr.* 287: 291, 1936.
3. Euler, H. v., Albers, H., and Schlenk, F.: *Ztschr. f. physiol. Chem.* 240: 113, 1936.
4. Harden, A., and Young, W. J.: *Proc. Roy. Soc., London, s. B.* 77: 405, 1906.
5. Quastel, J. H., and Wheatley, A. H. M.: *Biochem. J.* 32: 936, 1938.
6. Elvehjem, C. A., Madden, R. J., Strong, F. M., and Woolley, D. W.: *J. Am. Chem. Soc.* 59: 1767, 1937.
7. Fouts, P. J., Helmer, O. M., Lepkovsky, S., and Jukes, T. H.: *Proc. Soc. Exper. Biol. & Med.* 37: 408, 1937.
8. Smith, D. T., Ruffin, J. M., and Smith, S. G.: *J. A. M. A.* 109: 2054, 1937.
9. Spies, T. D., Cooper, C., and Blankenhorn, M. A.: *J. A. M. A.* 110: 622, 1938.
10. Kohn, H. I.: *Biochem. J.* 32: 2075, 1938.
11. Lwoff, A., and Lwoff, M.: *Proc. Roy. Soc., London, s. B.* 122: 352, 360, 1937.
12. Kohn, H. I., and Bernheim, F.: *J. Clin. Investigation* 28: 585, 1939.
- 12a. Axelrod, A. E., Gordon, E. S., and Elvehjem, C. A.: *Am. J. M. Sc.* 199: 697, 1940.
13. Vilter, R. W., Vilter, S. P., and Spies, T. D.: *J. A. M. A.* 112: 420, 1939.
14. Kohn, H. I., and Klein, J. R.: *J. Biol. Chem.* 130: 1, 1939.
15. Vilter, R. W., Vilter, S. P., and Spies, T. D.: *Nature* 144: 943, 1939.
16. Kohn, H. I.: Unpublished experiments.
17. Kohn, H. I., Klein, J. R., and Dann, W. J.: *Biochem. J.* 33: 1432, 1939.
18. Axelrod, A. E., Madden, R. J., and Elvehjem, C. A.: *J. Biol. Chem.* 131: 85, 1939.

19. Vilter, R. W., Vilter, S. P., and Spies, T. D.: *Am. J. M. Sc.* 197: 322, 1939.
20. Euler, H. v., Schlenk, F., Heiwinkel, H., and Högberg, B.: *Ztschr. f. physiol. Chem.* 256: 208, 1938.
21. Bernheim, F., and Felsovanyi, A. v.: *Science* 91: 76, 1940.

Vitamin A:

1. Bloch, C. E.: *J. Hyg.* 19: 283, 1921.
2. Fridericia, L. S., and Holm, E.: *Am. J. Physiol.* 73: 63, 1925.
3. Holm, E.: *Am. J. Physiol.* 73: 79, 1925.
4. Yudkin, A. M., Kriss, M., and Smith, A. H.: *Am. J. Physiol.* 97: 611, 1931.
5. Sie, B. L.: *Arch. f. Augenh.* 110: 610, 1937.
6. Jeghers, H.: *Ann. Int. Med.* 10: 1304, 1937.
7. Hecht, S., and Mundelbaum, J.: *J. A. M. A.* 112: 1910, 1939.
8. Wald, G., Jeghers, H., and Arminio, J.: *Am. J. Physiol.* 123: 732, 1938.
9. Wald, G.: *J. Gen. Physiol.* 21: 795, 1938.
10. Wald, G., and Clark, A.: *J. Gen. Physiol.* 21: 93, 1937.
11. Janesio, N. V., and Janesio, H. V.: *Biochem. Ztschr.* 287: 289, 1936.
12. Wald, G.: *J. Gen. Physiol.* 22: 391, 1939.
13. Wald, G.: *Nature* 140: 515, 1937.
14. Haig, C., Hecht, S., and Patek, A. J., *Science* 87: 534, 1938.
15. Yudkin, A. M.: *Arch. Ophth.* 19: 366, 1938.

Eserine (Physostigmine), Prostigmine, and Morphine:

1. Dale, H. H.: *Cold Spring Harbor Symposia* 4: 143, 1936.
2. Brown, G. L.: *Physiol. Rev.* 17: 485, 1937.
3. Cannon, W. B.: *Science* 90: 521, 1939.
4. Feldberg, W., and Vartiainen, A.: *J. Physiol.* 81: 39, 1934.
5. Cannon, W. B., and Rosenblueth, A.: *Am. J. Physiol.* 119: 221, 1937.
6. Kahane, E., and Lévy, J.: *Bull. Soc. chim. biol.* 18: 529, 1936.
7. Kahane, E., and Lévy, J.: *Compt. rend. Soc. de biol.* 125: 252, 1937.
8. Schweitzer, A., and Wright, S.: *J. Physiol.* 89: 165, 1937.
9. Myerson, A., and Thau, W.: *Arch. Ophth.* 18: 78, 1937.
10. Schweitzer, A., and Wright, S.: *Quart. J. Exper. Physiol.* 28: 33, 1938.
11. Baq, Z. M., and Brown, G. L.: *J. Physiol.* 89: 45, 1937.
12. Zimmerman, M., Frank, R., and Necheles, H.: *Arch. Surg.* 33: 188, 1936.
13. Henderson, W. R., and Wilson, W. C.: *Quart. J. Physiol.* 26: 83, 1936.
14. Walanski, J., and Zawadzki, R.: *Acta. Biol. Exper. (Warsaw)* 12: 119, 1938.
15. Clark, A. J., and Raventos, J.: *Quart. J. Exper. Physiol.* 28: 155, 1938.
16. Foster-Kennedy: *J. A. M. A.* 110: 198, 1938.
17. Russell, W. R., and Stedman, E.: *Lancet* 131: 742, 1936.
18. Bernheim, F., and Bernheim, M. L. C.: *J. Pharmacol.* 57: 427, 1936.
19. Silver, G. A., and Morton, H. G.: *J. Pharmacol.* 56: 446, 1936.
20. Kuhn, H. H., and Surles, D.: *Arch. internat. de pharmacodyn. et de therap.* 58: 88, 1938.
21. Slaughter, D., and Gross, E.: *J. Pharmacol.* 68: 96, 1940.
22. Slaughter, D., and Mimsell, D. W.: *J. Pharmacol.* 68: 104, 1940.
23. Nachmansohn, D.: *Compt. rend. Soc. de biol.* 130: 1065, 1939.

Amines, Adrenaline, and Sympathomimetic Drugs:

1. Hare, M. L. C.: *Biochem. J.* 22: 968, 1928.
2. Bernheim, M. L. C.: *J. Biol. Chem.* 93: 209, 1931.
3. Bernheim, F., and Bernheim, M. L. C.: *J. Biol. Chem.* 123: 317, 1938.
4. Slotka, K. H., and Müller, J.: *Ztschr. Physiol. Chem.* 238: 14, 1936.
5. Pugh, C. E. M., and Quastel, J. H.: *Biochem. J.* 31: 286, 1937.
6. Blaschko, H., Richter, D., and Schlossmann, H.: *Biochem. J.* 31: 2187, 1937.
7. Blaschko, H., Richter, D., and Schlossmann, H.: *J. Physiol.* 90: 1, 1937.
8. Bonhomme, F.: *Arch. internat. de physiol.* 43: 341, 1936.
9. Smith, R. G.: *Arch. f. exper. Path. u. Pharmacol.* 187: 604, 1937.
10. Lissak, K.: *Science* 87: 371, 1938.
11. Ito, S.: *Folia pharmacol. Japon.* 22: 137, 1936.
12. Welch, A. D.: *Am. J. Physiol.* 108: 360, 1934.
13. Kohn, H. I.: *Biochem. J.* 31: 1693, 1937.
14. Richter, D.: *Biochem. J.* 31: 2022, 1937.
15. Gaddum, J. H.: *J. Physiol.* 94: 87, 1938.
16. Blaschko, H.: *Nature* 145: 26, 1940.
17. Mann, P. J. G., and Quastel, J. H.: *Biochem. J.* 34: 414, 1940.
18. Philpot, F. J.: *J. Physiol.* 97: 301, 1940.
19. Green, D. E., and Richter, D.: *Biochem. J.* 31: 596, 1937.
20. Heirman, P.: *Compt. rend. Soc. de biol.* 127: 341, 1938.
21. Baq, Z. M.: *J. Physiol.* 92: 28, 1938.

Histamine:

1. Barger, G., and Dale, H. H.: *J. Physiol.* 41: 499, 1911.
2. Ackermann, D., and Mohr, M.: *Ztschr. f. physiol. Chem.* 255: 75, 1938.
3. Akiyama, Y.: *Fukuoka acta med.* 30: 42, 1937.
4. Schild, H.: *Quart. J. Exper. Physiol.* 26: 165, 1936.
5. Feldberg, W., and Kellaway, C. H.: *J. Physiol.* 90: 257, 1937.
6. Trethterwie, E. R.: *Australian J. Biol. & M. Sc.* 17: 145, 1939.
7. Feldberg, W., and Kellaway, C. H.: *J. Physiol.* 90: 280, 1937.
8. Feldberg, W., and Kellaway, C. H.: *J. Physiol.* 90: 288, 1937.
9. Code, C. F., and Hester, H. R.: *Am. J. Physiol.* 127: 71, 78, 1939.
10. Dragstedt, C. A., Eyer, S. W., and de Arellano, M. R.: *Proc. Soc. Exper. Biol. & Med.* 38: 641, 1938.
11. Bartelheimer, H., and Afendulis, T. C.: *Ztschr. f. d. ges. exper. Med.* 104: 31, 1938.
12. Bachmann, H.: *Arch. f. exper. Path. u. Pharmacol.* 190: 345, 1938.
13. Loeper, M.: *Bull. Acad. de méd., Paris* 120: 29, 1938.
14. Graham, J. R., and Wolff, H. G.: *Tr. Am. Neurol. A.* 63: 164, 1937.
15. Clark, D., Hough, H., and Wolff, H. G.: *Arch. Neurol. & Psychiat.* 35: 1054, 1939.
16. Rothberger, C. J., and Sachs, A.: *Quart. J. Exper. Physiol.* 29: 69, 1939.
17. Marcu, I.: *Compt. rend. Soc. de biol.* 130: 575, 1939.
18. Anrep, G. V., Barsoum, G. S., and Talaat, M.: *J. Physiol.* 86: 431, 1936.
19. Code, C. E., Evans, C. L., and Gregory, R. A.: *J. Physiol.* 92: 344, 1938.
20. Anrep, G. V., and Barsoum, G. S.: *J. Physiol.* 85: 409, 1935.
21. Katz, G.: *Science* 91: 221, 1940.
22. Rosenthal, S. R., and Minard, D.: *J. Exper. Med.* 70: 415, 1939.
23. Best, C. H., and McHenry, E. W.: *J. Physiol.* 70: 349, 1930.
24. McHenry, E. W., and Gavin, G.: *Biochem. J.* 26: 1365, 1932; 29: 622, 1935.
25. Edlbacher, S., and Zeller, E. A.: *Helvet. chim. acta* 20: 717, 1937.
26. Zeller, E. A.: *Helvet. chim. acta* 21: 1645, 1938.
27. Zeller, E. A., Schär, B., and Staehlin, S.: *Helvet. chim. acta* 22: 837, 1939.
28. Zeller, E. A., and Schär, B.: *Schweiz. med. Wchnschr.* 68: 1318, 1938.
29. Holtz, P., Heise, R., and Sprever, W.: *Arch. f. exper. Path. u. Pharmacol.* 188: 580, 1938.
30. Haag, F. E., and Latz, L.: *Ztschr. Immunitätsforsch. u. exper. Therap.* 96: 117, 1939.
31. Albus, G.: *Klin. Wchnschr.* 18: 858, 1939.
32. Foshay, L., and Hagebusch, O. E.: *J. A. M. A.* 112: 2398, 1939.
33. Prickman, L. E.: *J. A. M. A.* 112: 1102, 1939.
34. Roth, G., and Horton, B. T.: *Proc. Staff Meet., Mayo Clin.* 12: 7, 1937.
35. Baker, T. W.: *J. A. M. A.* 114: 1059, 1940.
36. Miller, H., and Piness, G.: *J. A. M. A.* 114: 1742, 1940.

Miscellaneous:

37. Bernheim, F., and Bernheim, M. L. C.: *J. Pharmacol.* 64: 209, 1938.
38. Michel, H. O., Bernheim, F., and Bernheim, M. L. C.: *J. Pharmacol.* 61: 321, 1937.
39. Quastel, J. H.: *Physiol. Rev.* 19: 135, 1939.

COENZYMES I AND II IN HUMAN BLOOD*

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INTRODUCTION

FOR many years the two dietary deficiency diseases, canine blacktongue and human pellagra, have been regarded as analogous syndromes. The belief that both of them might result from a deficiency of the same chemical substance or substances was supported by the fact that the symptoms of both diseases were relieved by the administration of liver extract.^{1,2} In 1937 Elvehjem, Madden, Strong, and Woolley³ isolated the amide of nicotinic acid from liver extract and reported that it was this substance in liver extract which cured blacktongue in dogs. Following this observation, nicotinic acid and, later, nicotinic acid amide were used clinically with spectacular success in the treatment of human pellagra.⁵⁻⁷

Naturally, the striking clinical results from the use of nicotinic acid and nicotinic acid amide in the treatment of pellagra have led to increased interest in the respiratory coenzymes I and II, cozymase and coferment,[†] respectively, which are known to contain nicotinic acid amide. These coenzymes are, by definition, specific organic catalysts which are relatively heat-stable, dialyzable, and which retain activity when separated from the living cell. Each is specifically necessary in an enzymatic reaction, and each is produced by living cells from nicotinic acid amide, ribose, adenylic acid, and phosphoric acid.

A general survey of the literature during the past thirty-six years shows notable advances in scientific knowledge dealing with coenzymes I and II. Harden and Young (1906)⁸ found that a dialyzable fraction of boiled yeast juice stimulated alcoholic fermentation. Later Meyerhof (1918)⁹ showed that these coenzymes occurred also in muscles and animal organs and that they could function in vitro to increase the respiration of fresh minced muscles. It was not until 1932, however, that nicotinic acid amide was isolated from the purest preparations of these coenzymes.^{10, 11} The present knowledge of the chemical constitution of coenzymes I and II indicates that these substances

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†In this paper, coenzyme I will be designated frequently as cozymase, and coenzyme II, as coferment. Other names for coenzyme I are diphosphopyridine nucleotide D.P.N., or codehydrogenase I; similarly, other names for coenzyme II are triphosphopyridine nucleotide T.P.N., or codehydrogenase II.

We are very grateful to Professor H. von Euler, Biochemiska Institutet, Stockholm, Sweden, for samples of pure cozymase; and to Herr Geheimrat Doctor Otto Warburg, Kaiser Wilhelm Institut für Zellphysiologie, Berlin-Dahlem, Germany, for pure coferment and pure cozymase.

differ chemically in their content of phosphoric acid, but are similar in that both are pyridine nucleotides which are indispensable for carbohydrate and protein metabolism.

The principles involved in the problem of the stimulation, cessation, or inhibition of cellular growth have been studied in a number of ways. It is perhaps wise to review briefly our present thoughts on this matter. We consider that the growth and respiration of cells, of which rapidly growing bacteria are excellent examples, involve the synthesis of complex substances from simpler compounds and the organization of these substances to form protoplasm. The pattern of growth is attended by numerous side reactions and energy changes which occur at body temperatures and therefore must be catalyzed. This physiologic catalysis can take place only with the aid of metallic ions, specific enzymes of a protein nature, and certain crystalloidal organic substances which work with the enzymes and are designated coenzymes. In many instances, coenzymes can be synthesized by lower forms of life as is true in the case of most strains of the dysentery bacillus, the growth of which can actually be used as a measure of the amount of nicotinic acid amide or nicotinic acid (coenzyme I and II precursors) which is present in the medium. Other organisms, such as the influenza bacillus, differ in that they require coenzyme I and II preformed in order to grow. Accordingly, we can obtain a measure of the enzymatic growth factor in tested material by bacterial growth, and thus obtain an index of the amount of the substance present, when, and only when, the specific bacterium is unable to synthesize the essential substances.

METHODS AND MATERIALS

Since the methods for studying coenzymes I and II in the blood are not simple and are not always satisfactory, several methods have been tried in this laboratory: (1) A specific biologic method for the determination of coenzyme, using yeast, "Koehsaft" (fluid obtained from blood and soft tissue after extraction with boiling water for two minutes), and a manometric apparatus;^{12, 13} (2) a chemical test for pyridine nucleotides, in which a colorimetric test for nicotinic acid amide is used before and after hydrolysis of the test material; by this method the increased amount of nicotinic acid amide found after hydrolysis can be calculated in terms of either coenzyme or coferment;¹⁴ (3) two bacteriologic methods using the growth properties of either *B. influenzae* or *B. parainfluenzae*^{15, 16} for the estimation of the total concentration of coenzymes I and II and possibly of unknown substances with similar properties.

A bacteriologic method¹⁵ has been used chiefly by us because it seemed that the principles concerned with the metabolism of these living bacterial cells were similar to those which govern the metabolic changes in the cells of the human body. The following detailed information concerns the method as originally published,¹⁵ with a number of modifications.

The Reproducibility of the Method.—The reproducibility of the method has been tested by making from six to ten determinations on the same blood, using the same medium and culture. The dilutions of blood in broth varied from 1:1,000 to 1:12,000 as has been reported previously,¹⁵ and the series of dilutions included several dilutions closely spaced near a previously determined

end point of growth. In these tests, the growth regularly stopped at the same dilution, as was determined by several observers, and the sharp end point occurred where the dilutions of blood were from 10 to 15 per cent apart. Therefore, the largest error possible was ± 15 per cent.

Computations Based on Visual Comparisons.—Along with the series of experiments described above, standards were prepared from ten concentrations of von Euler's purest cozymase (0.01 to 0.003 $\mu\text{g.}$ per cubic centimeter in the basal medium), and the tubes were inoculated from the same culture. After a twenty-four-hour period of incubation, the tubes containing the cozymase standards and the test determinations on blood were examined visually before a direct light. The tube that contained the highest dilution of blood which supported visible growth was matched with the tube containing cozymase standard most nearly identical in optical density. The blood dilutions factor, e.g., 8,000, was multiplied by the concentration of cozymase, e.g., 0.005 $\mu\text{g.}$ per cubic centimeter, in the selected standard, and the result expressed as cozymase equivalents per cubic centimeter of whole blood (40 $\mu\text{g.}$ per cubic centimeter). Next, from five to ten determinations were made on blood samples from seven normal persons, and the results were compared with a cozymase standard as before. The average error of these determinations was ± 8 per cent.

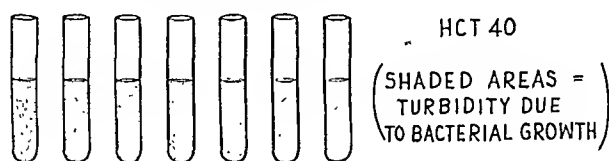
The term "cozymase equivalents" has been used¹⁷ to indicate that all the coenzyme-like substances in blood which function as factor V for *Hemophilus parainfluenzae* can be calculated arbitrarily as cozymase equivalents. We have adopted this estimation for the determination of coenzymes I and II with the influenza bacillus. This calculation is useful, but the proof that coenzymes I and II can act interchangeably in all reactions and to the same extent as growth-limiting factors (factor V) for *H. parainfluenzae* and *H. influenzae* is inadequate; that is to say, in the proper medium (lacking only these coenzymes), and within certain ranges of concentration, the extent of growth (or more properly the rate of reproduction) of a twenty-four-hour culture of either of these organisms is dependent upon the quantity of either coenzyme, I or II, that is present.

Recovery of Cozymase.—In assaying the recovery of cozymase, the end point of growth in a normal blood was determined first in the routine manner. Twenty-four hours later another sample of the same blood was tested. The procedure for each sample was to prepare three laked solutions of the blood; the first was deproteinized¹⁸ without any further additions; to a second, 10 or 20 $\mu\text{g.}$ of cozymase were added just before the sample was deproteinized; and to a third, 10 or 20 $\mu\text{g.}$ of cozymase were added after the deproteinization. In six series of determinations where the dilutions near the estimated end point of growth were from 10 to 20 per cent apart, the end point of growth was the same whether the cozymase was added before or after deproteinization. The added cozymase which was recovered from different visual or photometric determinations varied from 80 to 95 per cent with several samples of cozymase.

Calculations Based on Photometric Comparisons.—As in the visual comparison of bacterial densities, photometric studies of the relation of the growth of *B. influenzae* in autoclaved hemopectone broth (which contains added hematin) have shown that only in a limited range of concentrations is the

growth proportional to the concentration of pure cozymase added. Even though there is blood present in the basal autoclaved medium, other factors affect the density of bacterial growth since large concentrations of fresh blood support a more luxuriant growth in the autoclaved blood-peptone broth than does an excess quantity of cozymase. In the higher dilutions of blood which support growth, however, the influence of these secondary factors appears minimal. Therefore, we consider the end point of growth to be a most sensitive test for the coenzymes (Fig. 1). As was suggested by Kohn¹⁶ for his standard yeast extracts, we have found that photometric comparison of the density of bacterial growth in dilution of blood with the turbidity of bacteria grown in

**BLOOD OF A NORMAL PERSON SUPPORTS GROWTH
OF *B. INFLUENZAE* TO $\frac{1}{6000}$ OR $\frac{1}{8000}$ IN
HEMOPEPTONE BROTH**



**BLOOD OF A CLINICAL PELLAGRIN SUPPORTS
GROWTH OF *B. INFLUENZAE* TO $\frac{1}{2000}$ OR $\frac{1}{4000}$**

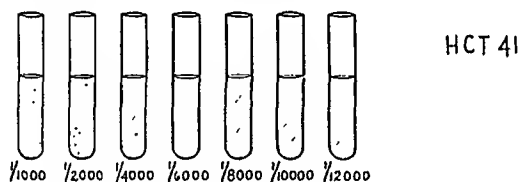


Fig. 1.—Each row of tubes shows the sharp end point of the growth of *B. influenzae* which is supported by dilutions of blood in autoclaved hemopeptone broth. The first row of tubes contains aliquot portions of blood from a "normal" person, and the second row, comparable portions of blood from a patient with clinical pellagra.

standard cozymase solutions (0.01 to 0.003 μ g. per cubic centimeter of basal medium) has simplified the determinations and recoveries of the coenzyme equivalents in blood. A curve of the optical densities plotted against the concentration of the standard cozymase solution is of value, however, only for the day of the test, for the culture of *B. influenzae* varies slightly from day to day. We recommend either the preparation of two cozymase standards* each day to compare with points on a concentration curve so that the value of the cozymase equivalents in a dilution of blood which supports *B. influenzae* can be interpolated from the curve of turbidities and multiplied by the dilution factor to obtain the cozymase equivalents per cubic centimeter of blood, or a determination on "normal" blood. In the latter case one can use the calibration curve only for relative comparison.

Normal Values.—Values obtained in normal blood for arbitrary cozymase equivalents, determined by the two bacteriologic methods of estimation, are of

*At the suggestion of Dr. A. E. Axelrod, the standards were kept frozen, and it was found that under such conditions, they were more constant than at the temperature of the refrigerator.

TABLE I

VALUES OF COENZYMES I AND II IN BLOOD OF NORMAL PERSONS

COZYMASE EQUIVALENTS (μ g.)		COZYMASE EQUIVALENTS (μ g.)	
PER C.C. WHOLE BLOOD	PER C.C. RED BLOOD CELLS	PER C.C. WHOLE BLOOD	PER C.C. RED BLOOD CELLS
30	71	37	84
30	61	36	81
37	83	24	60
27	64	33	74
33	74	31	62
40	90	30	66
33	78	31	70
37	83	21	52
27	86	21	60
50	109	25	65
25	63	31	73
30	71	29	69
27	64	37	87
31	69	30	60
27	63	32	75
31	73	27	61
31	69	30	66
27	63	32	75
31	73	27	61
29	63	33	67
37	82		
32	70		
28	61		

"Normal" average of cozymase equivalents per cubic centimeter of whole blood = 31 μ g., or 3.1 mg. per cent.

"Normal" average of cozymase equivalents per cubic centimeter of packed cells = 69 μ g., or 6.9 mg. per cent.

somewhat similar order of magnitude." The values obtained here are remarkably consistent for any individual blood, using any single cozymase standard. We have found by the dilution and end point method and by photometric comparison of cozymase standards with the growth in higher dilutions of blood that normal values range between 20 and 50 μ g. of cozymase equivalents per cubic centimeter of normal blood, and between 55 and 85 μ g. per cubic centimeter of packed cells. The normal values, shown in Table I, have been obtained by photoelectric comparison in the Evelyn colorimeter of the growth of *B. influenzae* in accurately prepared serial dilutions of blood and in aliquots of a standard cozymase solution. The higher dilutions were used for the calculations, to minimize the effect of other growth-stimulating factors in fresh blood. Blood from a normal person was tested under identical conditions as a control for the experimental determinations.

CLINICAL APPLICATIONS

1. *Pellagra*.—In 1939, at the Hillman Hospital Nutrition Clinic, coenzyme studies with a bacteriologic method¹⁵ were made upon 145 selected cases of pellagra. Similar studies have been made upon 45 patients so far in 1940. Most of the subjects were ambulatory and subsisted on the deficient diets which are typical of the pellagrins in this clinic. For the purpose of special observations, some of these patients were hospitalized and maintained on glucose and water. These cases were selected for study because it was believed that they were deficient in nicotinic acid and might be lacking in the dinucleotides, co-

enzymes I and II, which contain nicotinic acid amide. In these studies, it was observed that the degree of the deficiency of coenzymes I and II in whole blood and in packed cells did not parallel the severity of any one of the pellagrous symptoms, but in general paralleled the clinical state of the patient. There were, however, a few noteworthy exceptions. Table II shows the results of the initial assay of 45 clinical and subclinical cases of pellagra and indicates that in pellagrins there is a decrease in the content of the coenzyme growth factor in the whole blood. In the anemic patients, there frequently is an apparent compensation for the lack of cells, so that the total coenzyme value per cubic centimeter of cells is much higher than in normal red blood cells (Fig. 2).

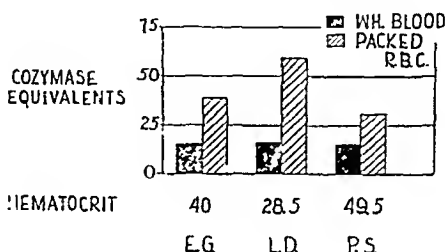


Fig. 2.—Relation of cozymase equivalents in whole blood and in packed cells. Figs. 2, 6, and 7 illustrate the various ways of considering the cozymase equivalents in blood. In this figure, the cozymase equivalents in the whole blood of three persons were essentially the same, whereas the percentage of packed cells varied in the three cases. Fig. 6 shows three cases where there was parallelism in the content of cozymase equivalents in whole blood and in the percentage of packed cells. In contrast, Fig. 7 shows that, with the same percentage of red blood cells in the blood of three persons, the cozymase equivalents in whole blood may vary considerably. These findings suggest that it is best to consider the coenzyme content of the blood as a functional unit rather than to set up arbitrary standards which may not always correlate with any particular determination of the formed elements of the blood.

In many of our mild cases the results are consistent with the results of Kohn and Bernheim,¹⁷ who found that, in nine cases of pellagra, the concentration of cozymase equivalents in packed cells was "only moderately low."* But if one compares the results in Table II with those in Table I, he sees that the concentration of the coenzymes in the blood of persons with optimal nutrition is consistently higher. It is to be noted also that nicotinic acid therapy produces clinical improvement, that it never fails to increase the content of the blood coenzymes in persons with uncomplicated pellagra, and that the ingestion of nicotinic acid by normal persons also increases the concentration of these substances in the blood.

2. *Diabetes Mellitus*.—Some years ago we learned that the administration of large amounts of glucose over a period of weeks to chronic pellagrins predisposed many of them to a recurrence of their disease. This observation, together with the knowledge that patients with diabetes mellitus not infrequently have pellagra, suggested that the metabolism of glucose is associated with the antipellagra factor and that patients with diabetes mellitus should be selected for a coenzyme study.

*Our experience with the culture of *H. parainfluenzae* has been limited to studies of normal and pellagrous blood. The particular culture of *H. parainfluenzae* for which we are indebted to Dr. Kohn is well suited to turbidity measurements in our hands, but the density of growth is proportional to the concentration of cozymase only in a limited range (0.01 to 0.003 $\mu\text{g.}$). This finding is similar to the observation which we have made using our culture of *H. influenzae*. For that reason we believe the highest dilution of blood used in this test to be the best index of the coenzyme content. As parallel studies using both methods have not always given comparable results, further investigations are in progress.

TABLE II

VALUES OF COENZYMES I AND II IN BLOOD OF PELLAGRINS

GROUP I: PATIENTS WITH "NORMAL" HEMATOCRIT (39-49)		GROUP II: PATIENTS WITH LOW HEMATOCRIT, HIGH COENZYME PER C.C. OF CELLS		GROUP III: PATIENTS WITH LOW HEMATOCRIT, "NORMAL" COENZYME PER C.C. OF CELLS		GROUP IV: PATIENTS WITH LOW HEMATOCRIT, LOW COENZYME PER C.C. OF CELLS	
COZYMASE EQUIVA- LENTS (μ g.)		COZYMASE EQUIVA- LENTS (μ g.)		COZYMASE EQUIVA- LENTS (μ g.)		COZYMASE EQUIVA- LENTS (μ g.)	
PER C.C. WHOLE BLOOD	PER C.C. RED BLOOD CELLS	PER C.C. WHOLE BLOOD	PER C.C. RED BLOOD CELLS	PER C.C. WHOLE BLOOD	PER C.C. RED BLOOD CELLS	PER C.C. WHOLE BLOOD	PER C.C. RED BLOOD CELLS
+22	47	+ +22	90	+ + +15	54	+ + + 9	32
+18	44			+ +21	60		
				+21	69	+ +13	41
+18	39	+ -37	95	+21	58		
+15	37			+17	48	+ 7	24
+17	36			+18	55		
+22	52	+ -27	78	+18	66	+11	26
+22	52	+ -24	105	+17	71		
+25	61	+ -19	78	+13	52	+ -15	46
+ -18	44						
+ -18	40			+ -17	58		
+ -22	53			+ -25	66	+ -13	33
+ -18	44			+ -14	49		
+ -22	52						
+ -18	40						
+ -28	64						
+ -30	67						
Ribo. 25	58						
Ribo. 10	35						
Ribo. 19	40						
Ribo. 10	22						
Ribo. 16	42						
B ₁ 27	64						
Avg. 19.2 μ g.	47 μ g.	25.8 μ g.	89 μ g.	18 μ g.	59 μ g.	11.3 μ g.	33.6 μ g.

Average cozymase equivalents of 45 cases of pellagra = 17.2 μ g. per cubic centimeter of whole blood, or 1.72 mg. per cent.

Average cozymase equivalents of 45 cases of pellagra = 53 μ g. per cubic centimeter of packed red blood cells, or 5.3 mg. per cent.

Ribo, riboflavin deficiency. B₁, thiamin deficiency. Nicotinic acid deficiency. +-, subclinical pellagra; +, mild relapse; ++, moderate relapse; + + +, severe relapse.

Ten cases of extreme diabetic acidosis, in addition to the three previously reported,¹⁸ were tested (1) before insulin therapy, (2) after insulin and glucose had been injected and the patients had regained consciousness, and (3) again after they were "regulated" on a diabetic diet without special vitamin preparations. The red blood cell count of these patients on admission ranged from 5,500,000 to 6,000,000 per cubic millimeter because of dehydration; the hematocrit values were above 50 per cent. In contrast, the coenzyme content of the blood was low on admission, with an average limit of growth in a dilution of 1:1,000. When the patients were "regulated" as to diet and insulin requirement, the red blood cell counts and hematocrit readings were lower, and the coenzyme I and II content of the whole blood was increased so that the average limit of growth after successful regulation of the same seven patients was 1:6,000. Kohn and Bernheim¹⁷ have reported partial confirmation of this observation as a result of their study of 23 regulated diabetic persons not in a state of acidosis. In a more recent study, Sydenstricker, Geeslin, and Weaver¹⁹

have shown clinically that a very delicate balance exists in certain diabetic persons who have subsisted for long periods of time on inadequate and unbalanced diets and who have frequent bouts of active pellagra. The use of increased quantities of insulin and glucose in these patients induced an exacerbation of their pellagrous symptoms.

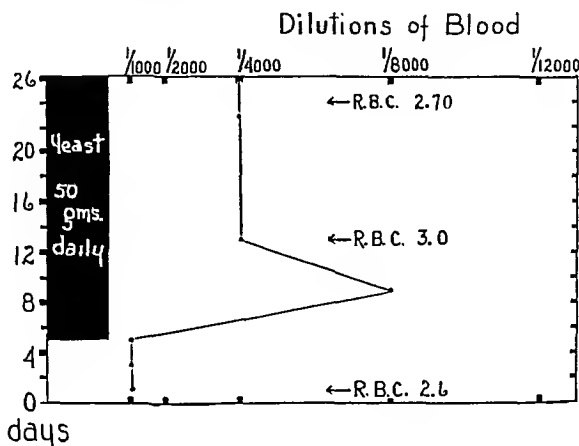


Fig. 3.—Maximum blood dilutions of a patient with chronic lymphatic leucemia supporting growth of *B. influenzae*. This figure illustrates the low values for the coenzyme I and II content of the blood of persons who have severe leucemia. This particular patient had a spectacular increase in the coenzyme content of blood following oral administration of yeast. This increase paralleled the patient's greater strength and sense of well-being, although there were no demonstrable changes in the number or character of the cellular elements as seen in blood smears.

3. *Leucemia*.—Studies of the blood coenzyme values of patients with lymphogenous and myelogenous leucemia have not led to conclusive or consistent results. In 23 of 27 cases studied by us, the coenzyme values were found to be lower than normal, as was described in an earlier study.²⁰ In four selected cases of lymphatic leucemia, nicotinic acid, administered orally or intravenously, repeatedly failed to increase the level of the coenzymes. In three of the four cases the daily simultaneous injection of 25 mg. of nicotinic acid and 5 mg. of riboflavin induced a slight, but temporary, increase in the coenzyme level, but the oral ingestion of from 60 to 100 Gm. of brewer's yeast daily increased and maintained the coenzyme level for weeks (Fig. 3). Of these 27 cases, one patient, H. G., was admitted to the hospital with moist sores around the lips of several weeks' duration. After riboflavin (5 mg. daily) was given intravenously for five days, the sores healed and never returned throughout a six weeks' period of observation. Another interesting case in this group was F. H., who, when admitted to the hospital in extremis, had coexisting clinical pellagra. These two persons were the only ones of the group of 27 who showed clinical evidence of a specific vitamin deficiency.

4. *Pneumococcal Pneumonia*.—A recent report²¹ of 20 cases of pneumococcal pneumonia, which were studied before and after serum or sulfapyridine therapy, has shown that 17 of the 20 patients studied had blood coenzyme values which were below the lowest normal limits found during a simultaneous study of 50 normal persons. The low values rose spontaneously to normal in these 17 patients following the crisis of their disease.

5. *Roentgen Sickness*.—The study of the coenzyme content of blood was extended to persons who experienced acute but temporary roentgen sickness, because it had been observed²² some time ago that people receiving therapeutic doses of roentgen rays excreted abnormal quantities of a porphyrin-like pigment after exposure to the radiation. Studies on 10 selected patients receiving courses of deep roentgen therapy have shown a rapid decrease within twelve hours in the coenzyme I and II content of whole blood which was not associated with any change in the hematocrit²³ (Fig. 4). Normal levels per cubic centimeter of blood were obtained, however, on these same patients from twenty-four to thirty-six hours after the exposure. Unpublished results of Bean, Vilter, and Spies have shown that roentgen radiation of blood in vitro did not cause a decrease in coenzyme content.

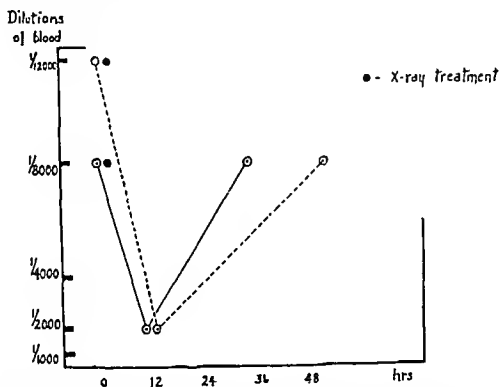


Fig. 4.—Showing the decrease in the coenzyme I and II concentration of the blood of two "normal" persons following roentgen therapy, as shown by the growth of *B. influenzae* in aliquot dilutions of blood in hemopectone broth.

DISCUSSION

It has been shown in two laboratories^{15, 16} that nicotinic acid or nicotinic acid amide, given orally or intravenously, increases the measurable concentration of coenzymes I and II in the blood of normal people and pellagrins. Along with the increase in the blood, there is a concomitant increase in the normal excretion of nicotinic acid and of coenzymes I and II in the urine. In this clinic, low values for coenzymes I and II have been observed in the blood of selected patients with pellagra, diabetes mellitus, acute pneumococcal pneumonia, and roentgen sickness (Table III). These findings suggest that the administration of vitamin B concentrates or nicotinic acid to persons with other acute infections or metabolic disturbances might well be evaluated clinically in a large number of cases. The use of nicotinic acid is accepted in the treatment of pellagra and has been suggested in certain cases of roentgen sickness^{22, 24} and untoward sulfanilamide reactions.²⁵

TABLE III

TWENTY-FOUR-HOUR GROWTH OF *B. INFLUENZAE* IN DILUTIONS OF BLOOD IN AUTOCLAVED PEPTONE-BLOOD BROTH

TYPICAL ROUTINE STUDIES ON BLOOD FROM VARIOUS PATIENTS		DILUTIONS OF BLOOD							R.B.C. (C.M.M.)	HEMA- TOCRIT
		1:1,000	1:2,000	1:4,000	1:6,000	1:8,000	1:10,000	1:12,000		
1.	A pellagrin	+++	++	-	-	-	-	-		40
	Same sample of blood + cozymase	++++	++++	+++	+++	+++	+++	+++		
	Patient after 500 mg. nico- tinic acid	++++	++++	+++	++	+	-	-		40
2.	Patient with pneumococcal pneumonia	+++	++	-	-	-	-	-	4.6	
	Patient 24 hr. after crisis	++++	+++	+++	++	+	-	-	4.0	
3.	Diabetic patient in coma	++							5.9	
	Same sample of blood + cozymase	++++	++++	+++	+++	+++	+++	+++		
	Patient after "regulation"	++++	+++	++	++	+	-	-	4.8	
4.	Patient with lymphatic leuce- mia	+							1.8	
	Same sample of blood + cozymase	++++	++++	+++	+++	++	++	++		
	After patient on: { 3 mg. ribo- flavin intra- venously 400 mg. nico- tinic acid orally 100 Gm. yeast orally } daily	+++	++	++	+	-	-	-	2.0	
5.	Patient before roentgen radi- ation	++++	+++	++	++	±	-	-	5.0	
	Same patient 6 hr. after ex- posure	++++	+	-	-	-	-	-	5.0	
	Same patient 30 hr. after ex- posure	++++	++++	++	++	+	±	-	5.0	
6.	Typical normal control	++++	++++	+++	+++	+	±	-	4.3	

The increase in coenzyme I and II content of the blood in patients with pellagra, pneumococcal pneumonia, diabetes mellitus, lymphatic leucemia and roentgen sickness following nicotinic acid, sulfapyridine, insulin and well-balanced diet, riboflavin and yeast, respectively. Controls were made, adding cozymase to the same samples of blood, and blood from normal persons was used as an additional control in every instance.

FACTORS WHICH OPERATE TO DISTURB NUTRITIONAL BALANCE AND TO AFFECT THE
COENZYME I AND II CONTENT OF THE TISSUES

External and internal causal factors associated with the development of dietary deficiencies are detrimental to the proper physiologic function of the body. The most frequent cause of nutritional deficiency disease is a decreased intake of vitamins and other nutritious substances. In addition, some persons develop deficiency diseases because of faulty assimilation, even though the dietary intake may be adequate. Particularly in these persons does the continuous excretion of nicotinic acid and other vitamins operate against their general well-being. Excessive exercise taxes their strength and appears to precipitate relapse and clinical manifestations of deficiency disease, whereas acute infection and fever place still greater demand for vitamins upon the body. It appears likely that some of the same influences operate against the

nutritional health of a "normal" person, but beneficial mechanisms act more efficiently and against lesser odds and thus succeed in maintaining the homeostatic equilibrium.

FACTORS WHICH OPERATE TO MAINTAIN NUTRITIONAL BALANCE

Even as there are conditions which upset nutritional equilibrium, there probably are as many factors which function to preserve the physiologic balance. Long before the etiology of pellagra was in any way understood, rest in bed was advocated as the best treatment for the disease. The effectiveness of this treatment is still observed, for a certain percentage of pellagrins in mild relapse, when given rest in bed without specific medication, return temporarily to apparent nutritional balance. Coenzyme I and II studies have been made on such cases, and it was found that the initial concentration of coenzymes I

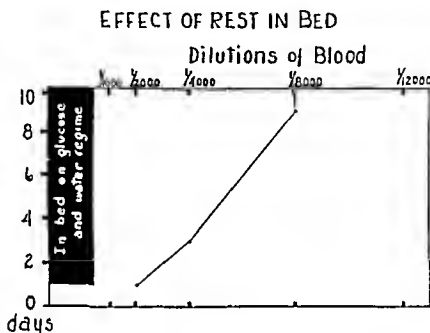


Fig. 5.—Showing the initial low coenzyme I and II content of the blood of a pellagrins who later showed an increase to normal levels following rest in bed and a caloric maintenance of glucose. This type of observation strikingly illustrates the necessity of considering the patient from a physiologic point of view.

and II per cubic centimeter of blood increased spontaneously along with the clinical improvement that occurred in 30 per cent of the patients who were hospitalized and maintained on glucose and water without specific therapy (Fig. 5). Similar phenomena occurred during the initial treatment of persons in diabetic coma, for no nutrients, other than saline and glucose, were given. Since it was thought that the presence of interfering substances had been ruled out by the addition of standard cozymase solution to blood control tubes, the observations suggested that in these cases there was sufficient storage of nicotinic acid and the coenzymes in various tissues. The observations of other workers seem to support this theory in certain particulars, for the studies of Axelrod and Elvehjem²⁶ and of Kohn, Klein, and Dann²⁷ on dogs with blacktongue, and the unpublished determinations of Axelrod on the cozymase content of skeletal muscle in human beings with pellagra in relapse have shown that the level of these coenzymes, particularly cozymase in the blood, brain, and kidney cortex, remains at a fairly high level in blacktongue, although it decreases in the liver and muscles of dogs with blacktongue and in the muscles of human beings

with pellagra. It seems that there is protective homeostatic control of the storage and distribution of these vital substances in the cells. The process may carry on for the benefit of some organs and at the expense of others, and the equilibration becomes more difficult as the stores of these coenzyme precursors are depleted.

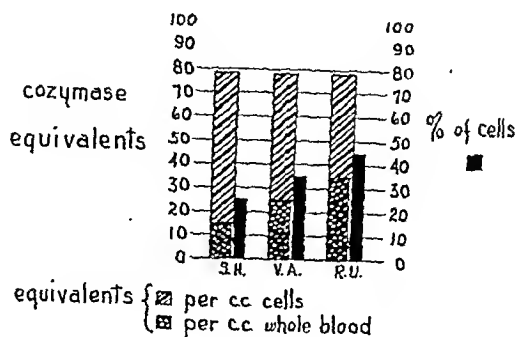


Fig. 6.

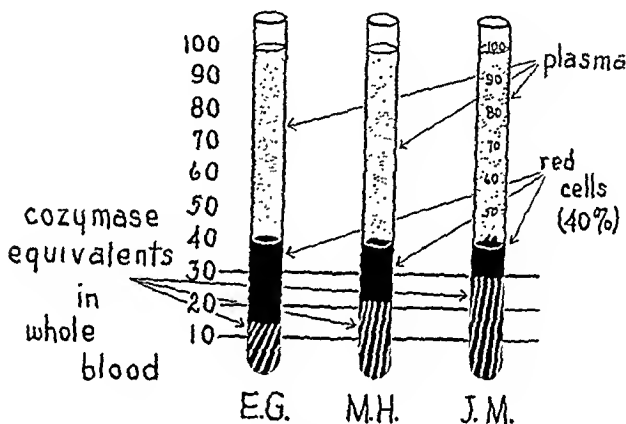


Fig. 7.

Figs. 6 and 7.—Variations in content of coenzyme equivalents of red blood cells.

FACTORS CONCERNED WITH THE CONCENTRATION OF COENZYMES IN WHOLE BLOOD AND PACKED CELLS

There has been considerable question as to the most precise method of expressing the units of concentration of these blood coenzymes, principally because of different theories of their formation and function. It has been suggested¹⁵ that the calculation should be based upon the concentration of coenzyme equivalents per eubic centimeter of packed cells since these coenzymes are largely intracellular. Cozyme equivalents, calculated in this manner, appear to compensate for differences in the total volume of red blood cells encountered during any clinical study. It presupposes a constancy of all factors other than the hematocrit values (percentage of cells per volume of blood) and assumes that coenzymes are synthesized and used only in the individual cells in which they are contained. It assumes also that the nicotinic acid amide in tissue fluids and the coenzymes of the cells are in constant equilibrium which is unaffected by changes in the number or size of the cells. Such a consideration would seem to exclude the possibility of any transfer of preformed coenzyme. The presence of these coenzyme-like substances in bacteria-free fresh urine, however, requires some explanation and further investigation of the extracellular existence of these substances. Studies of the excretion of these substances suggest either a diffusion of these coenzymes which may be protected in some way

from destruction by the nucleotidases of the serum, or synthesis and secretion of coenzymes by the cells of the kidney tubules, for normal, freshly voided, sterile urine contains about one to ten times as many coenzyme equivalents as does blood plasma. Little is known about the relative solubilities of the different antipellagric substances, their rates of diffusion, or their selective uptake by different types of cells. The *in vitro* synthesis of the coenzymes in whole blood from nicotinic acid or nicotinic acid amide^{28, 29} shows that some of these substances can enter cells, and it is known³⁰ that coenzyme is taken up from a medium within eight seconds by bacteria which require it for growth. The rate at which nicotinic acid amide or the coenzymes leave cells, however, is not known. The very small quantities of coenzymes in the serum suggest that many factors control the distribution.

An alternate method of expressing the concentration of these coenzymes in the blood is in units per cubic centimeter of whole blood. The reasons for this are several: (1) In anemias of uncomplicated blood loss, coenzyme equivalents per cubic centimeter of whole blood are usually within the normal range, while values per cubic centimeter of cells are usually high. (2) Some pellagrins with anemia have low values for the coenzyme equivalents in whole blood while these values per cubic centimeter of packed cells may be within the normal or high range. In contrast to this, pellagrins with normal hematocrit readings tend to have low values per cubic centimeter of cells as well as per cubic centimeter of whole blood (Table II). (3) There is no evidence that the coenzyme content of the red blood cell is proportional to cell size. It is labile and in most cases seems to be determined chiefly by the concentration of the nicotinic acid in the surrounding fluid, for it has been shown^{15, 16} that the red blood cell can almost double its coenzyme content following the ingestion of nicotinic acid. It appears to us that a calculation using the hematocrit reading as reference is based upon three or more variables: the concentration of coenzymes in the red blood cells, the size of the cells, and the number of red blood cells. These may vary independently and in direct or indirect proportion (Figs. 2, 6, 7).

SUMMARY AND CONCLUSIONS

1. In an extended study of mixed deficiency diseases, it has been observed that the concentration of the substances in whole blood which have the growth-stimulating properties of the pyridine nucleotides for the influenza and parainfluenza bacillus is lower in persons subsisting on deficient diets than in normal persons on optimal diets.

2. Low values for the coenzyme concentration of whole blood have been observed also in diabetes mellitus, leucemia, roentgen sickness, and pneumococcal pneumonia.

3. The concentration of these coenzymes in the blood of malnourished persons was decreased also by exacerbations of the deficiency symptoms, concurrent infections, and fever, or by excessive physical exercise. When these patients improved clinically either after rest in bed, improved dietary intake, or specific medication, a parallel increase in the concentration of these coenzymes in the blood was observed.

4. These studies suggest indirectly that coenzymes I and II in the blood are in homeostatic equilibrium with the acid amide and the same coenzymes in the other tissues of the body.

REFERENCES

1. Goldberger, J., and Sebrell, W. H.: Blacktongue Preventive Value of Minot's Liver Extract, *Pub. Health Rep.* 45: 3064, 1930.
2. Wheeler, G. A., and Sebrell, W. H.: The Control of Pellagra, *J. A. M. A.* 99: 95, 1932.

3. Spies, T. D.: Treatment of Pellagra by Means of Parenteral Liver Extract, *Proc. Soc. Exper. Biol. & Med.* 31: 363, 1933.
4. Elvehjem, C. A., Madden, R. J., Strong, F. M., and Woolley, D. W.: The Isolation and Identification of the Anti-Black Tongue Factor, *J. Biol. Chem.* 123: 137, 1938.
5. Fouts, P. J., Helmer, O. M., Lepkovsky, S., and Jukes, T. H.: Treatment of Human Pellagra With Nicotinic Acid, *Proc. Soc. Exper. Biol. & Med.* 37: 405, 1937.
6. Spies, T. D., Cooper, Clark and Blankenhorn, M. A.: The Use of Nicotinic Acid in the Treatment of Pellagra, *J. A. M. A.* 110: 622, 1938.
7. Schmidt, H. L., Jr., and Sydenstricker, W. P.: Nicotinic Acid in the Prevention of Pellagra, *J. A. M. A.* 110: 2065, 1938.
8. Harden, A., and Young W. J.: The Alcoholic Ferment of Yeast Juice. II. The Coferment of Yeast Juice, *Proc. Roy. Soc., London, s. B.* 78: 369, 1906.
9. Meyerhof, O.: Presence of the Coenzyme of the Alcoholic Yeast Fermentation in Muscle Tissue and Its Significance in the Respiratory Mechanism, *Ztschr. f. physiol. Chem.* 101: 165, 1918; *J. Chem. Soc.* 114: I, 242, 1918.
10. Warburg, O., Christian, W., and Griese, A.: Hydrogen-Transferring Coenzyme; Its Composition and Mode of Action, *Biochem. Ztschr.* 282: 157, 1935.
11. Von Euler, H., Albers, H., and Schlenk, F.: Cozymase, *Ztschr. f. physiol. Chem.* 237: I, 1935.
12. Von Euler, H., Mjörback, K., and Nilsson, R.: New Results on the Enzymic Decomposition of Carbohydrates, *Ergebn. d. Physiol.* 26: 531, 1928.
13. Von Euler, H., and Nilsson, R.: Cozymase Determination in the Blood, *Ztschr. f. physiol. Chem.* 162: 63, 1926.
14. Von Euler, H., Schlenk, F., Melzer, L., and Höglberg, B.: Nicotinic Acid Amide and Cozymase in Normal and in Avitaminotic Rats, *Ztschr. f. physiol. Chem.* 258: 212, 1939.
15. Vilter, R. W., Vilter, S. P., and Spies, T. D.: Relationship Between Nicotinic Acid and a Codehydrogenase (Cozymase) in Blood of Pellagrins and Normal Persons, *J. A. M. A.* 112: 420, 1939.
16. Kohn, Henry: The Concentration of Coenzyme-like Substance in Blood Following the Administration of Nicotinic Acid to Normal Individuals and Pellagrins, *Biochem. J.* 32: 2075, 1938.
17. Kohn, Henry I., Bernheim, Frederiek, with technical assistance of Felsovanyi, A. V.: The Blood V-Factor (Coenzyme) Level in Normal and Pathological Subjects, *J. Clin. Investigation* 18: 585, 1939.
18. Vilter, R. W., Vilter, S. P., and Spies, T. D.: Determination of the Codehydrogenases I and II (Cozymase) in the Blood of Diabetics in Severe Acidosis, *Am. J. M. Sc.* 197: 322, 1939.
19. Sydenstricker, V. P., Geeslin, L. E., and Weaver, G. W.: Avitaminosis Occurring in Diabetic Patients Under Insulin Therapy, *J. A. M. A.* 113: 2137, 1939.
20. Vilter, R. W., Vilter, S. P., and Spies, T. D.: A Note on the Blood Codehydrogenases I and II in Lymphatic or Myelogenous Leukemia, *South. M. J.* 32: 619, 1939.
21. Vilter, R. W., Bean, W. B., Rueggsegger, J. M., and Spies, T. D.: The Role of Coenzymes I and II in Blood of Persons With Pneumococcal Pneumonia, *J. Lab. & Clin. Med.* 25: 897, 1940.
22. Spies, T. D., and Bean, W. B.: The Role of Nicotinic Acid in the Prevention of Pellagra, Roentgen Sickness and Increased Porphyrinuria, *J. Clin. Investigation* 17: 504, 1938 (abst.).
23. Bean, W. B., Vilter, R. W., and Spies, T. D.: The Effect of Roentgen-Ray on the Blood Codehydrogenases I and II, *Ann. Int. Med.* 13: 783, 1939.
24. Graham, J. W.: Radiation Sickness, Treatment With Nicotinic Acid, *J. A. M. A.* 113: 664, 1939.
25. McGinty, A. P., Lewis, G. T., and Holtzclaw, M. R.: Symptoms Occurring With Sulfanilamide Relieved by Nicotinic Acid, *J. M. A. Georgia* 28: 6, 1939.
26. Axelrod, A. E., and Elvehjem, C. A.: The Determination of Coenzyme I in Animal Tissues, *J. Biol. Chem.* 131: 77, 1939.
27. Kohn, H. I., Klein, J. R., and Dann, W. J.: The V-Factor Content and Oxygen Consumption of Tissues of the Normal and Blacktongue Dog, *Biochem. J.* 33: 1432, 1939.
28. Kohn, H. I., and Klein, J. R.: The Synthesis of Cozymase and of Factor V From Nicotinic Acid by the Human Erythrocyte in Vitro and in Vivo, *J. Biol. Chem.* 130: 1, 1939.
29. Vilter, S. P., Vilter, R. W., and Spies, T. D.: Synthesis of Coenzymes I and II, *Nature* 144: 943, 1939.
30. Lwoff, André, and Lwoff, Marguerite: Studies on Codehydrogenases. I. Nature of Growth Factor "V," *Proc. Roy. Soc., London, s. B.*, No. 828 122: 352, 1937; II. Physiological Function of Growth Factor "V," *ibid.* 122: 360, 1937.

CLINICAL LABORATORY DETERMINATION OF VITAMIN NUTRITION*

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IT IS impossible, in a short article, to cover all the phases of clinical laboratory determination of vitamin nutrition, to give anything approaching a complete bibliography of the subject, or to discuss in detail controversial matters. With the introduction of simple methods for analysis of ascorbic acid, clinical laboratory diagnosis of vitamin nutrition began to be possible and its importance is increasingly recognized. Scurvy was formerly considered a rare disease. It has been surprising to find how many people have blood levels of ascorbic acid in or near the range at which clinical scurvy occurs. Likewise, when attention is paid to the symptoms which have been known to occur in classical pellagra and to the lesser objective manifestations of it, the number of patients whose symptoms may be alleviated by the administration of nicotinic acid or the vitamin B complex has been remarkable.¹

Our position in respect to vitamin deficiencies is now much the same as that of clinicians in respect to thyroid disease before clinical observations could be checked by basal metabolic rate determinations. The more extreme deficiencies usually can be recognized, but for each one of these there are, presumably, many cases of less apparent deficiency disease causing ill health.

The development of means of laboratory diagnosis of deficiency for many vitamins does not now appear to be as simple a problem as it has been for vitamin C. It may be expected, however, that means will be found. Then many cases of ill health of obscure cause may be explained. Many patients who are now commonly called psychoneurotics may receive a different diagnosis and more appropriate treatment. The lesser symptoms of deficiency disease may become familiar enough so that patients may commonly be given appropriate supplements without laboratory tests. More will be learned of the effects of vitamin deficiency on body function, such as the parallelism between the ascorbic acid and the complement activity of the blood observed by Ecker and Pillemer² in two scorbutic patients during treatment. The levels of vitamin nutrition beyond which further improvement will not benefit the individual may be determined.

Chemical and biologic methods have been developed for the assay of certain vitamins in the body fluids. The first chemical method was based upon the blue color which develops when vitamin A reacts with antimony trichloride in chloroform solution, observed by Carr and Price³ in 1926. This was first used for the determination of vitamin A in fish oils. The earlier analyses of vitamin A in blood serum were unreliable because of inadequate methods of extraction and separation from interfering substances. Von Eckelen and Emmerie⁴ in 1935

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introduced the important step of heating serum with potassium hydroxide before extracting it. This was used in the extensive studies of Lindqvist.⁵ Dann and Evelyn⁶ in 1938 used the Evelyn photoelectric colorimeter to permit reading of the blue color at its maximum and thereby avoid errors due to the waxing and waning of the color. Kimble⁷ described procedures to permit the use of this determination on small amounts of serum.

Ascorbic acid was the next vitamin to be measured by chemical analysis. Zilva in 1927⁸ reported that there was in fresh fruit juices a substance that would reduce phenolindophenol which was closely associated with the vitamin but was not, he thought, identical with it. Tillmans⁹ was able to eliminate the apparent discrepancies which Zilva had reported and established that vitamin C was a reducing substance. On the basis of extensive studies on oxidation-reduction potentials of dyes by Clark, Gibbs and associates¹⁰ he chose to use 2, 6-dichlorophenolindophenol which has continued to be used. Other chemicals have been used for titration but have not found much favor.

Because other constituents of body fluids are also reducing substances, the reaction is not specific for ascorbic acid. In blood other reducing substances are present in small amounts and cause reduction slowly. Consequently, by proper speed and choice of end point satisfactory results may be obtained with blood by the titration methods of Farmer and Abt,¹¹ and Pijoan and Klemperer,¹² which have been used extensively in this country.* In urine, however, Evelyn, Malloy, and Rosen,¹⁴ and Roe and Hall¹⁵ report that a large part of the reducing capacity is due to substances other than ascorbic acid. Previous studies of urinary ascorbic acid by titration methods, accordingly, need re-evaluation.

Mindlin and Butler¹³ described a method using the photoelectric colorimeter which eliminates the error due to other reducing substances. Using a larger quantity of dye than will be reduced, the residual color is read at thirty seconds. Correction for other reducing substances may be made by extrapolation to zero time from subsequent readings. Bessey¹⁶ added the further step of dropping in a crystal of ascorbic acid after the determination to complete the reduction of the dye and obtain a subsequent blank.

The status of chemical methods for vitamin B₁ determination is less completely established. The thiochrome reaction, which permits measurement of the fluorescence of partially oxidized thiamin, first observed by Peters,¹⁷ studied further by Jansen,¹⁸ and applied to the analysis of urine by Westenbrink and Goudsmit¹⁹ is capable of detecting small amounts of thiamin. With it there are possibilities of error which we have pointed out²⁰ and which are evidenced by the variety of procedures used in the attempt to avoid them and the wide variation in the range of values obtained by the different methods. The thiochrome method will be an asset to the clinical laboratory if reasonably accurate results may be obtained without too time-consuming procedure.

We have worked out a method^{20, 21} based on the reaction of thiamin with p-aminoacetophenone reported by Prebluda and McCollum²² which is highly specific and accurate. With urine, the necessity of concentration of the vitamins into a small volume and its separation from substances which inter-

*See Table I of Mindlin and Butler.¹³

fere with the reaction makes the test time-consuming. Hence the method is suited to the research laboratory and for the routine analysis of materials with a high content of the vitamin.

Meicklejohn²³ used its growth-promoting effect on *Phycomyces blakesleeanus* to measure thiamin in blood. Sinclair²⁴ has shown that the increment of growth is not specific for thiamin and has proposed to neutralize the influence of other substances by the addition of whole blood to the medium. Goodhart and Sinclair²⁵ have also measured by a fermentation method cocarboxylase in blood, where it comprises the major fraction of thiamin.

Before analyses of vitamins in the body fluids are clinically useful, it must be shown that the figures obtained reflect the level of vitamin nutrition in the tissues. In the case of ascorbic acid, blood values apparently do this. Van Eckelen²⁶ reported a straight line relationship between blood levels and the doses required for saturation. This deserves further investigation, but a correlation between intake and blood levels of ascorbic acid has been observed repeatedly.

We²⁷ have observed a close correlation between dietary history and urinary excretion of thiamin. Our studies²⁸ on experimental thiamin deficiency indicate that urinary excretion falls off very promptly when an inadequate diet is taken, and that the excretory response to a test dose is then needed to evaluate the body stores. On the other hand, when a person who has been depleted is given a normal intake of thiamin, the depleted state of the body stores continues to be shown by a low urinary excretion for a long time. In a large number of determinations of the blood cocarboxylase in a large variety of conditions Goodhart and Sinclair²⁹ obtained deviations from normal in the expected directions. The wide range of values which they found in the same normal individuals at different times indicates the necessity of taking blood samples at a constant, longer time after meals or a large possibility of error in the method.

Relatively little study has been made of riboflavin in human body fluids. Sebrell³⁰ and Sydenstricker³¹ and their associates have recently described manifestations of riboflavin deficiency which we now find to be fairly common. Ferrebee³² has recently described methods for measuring riboflavin in urine, in which the optical system is a considerable improvement over that of previous methods and for which the reported comparative results indicate good specificity and accuracy. The findings of an early drop in urinary excretion of riboflavin during experimental deficiency in rats reported by Vivanco³³ and in human beings by Emmerie,³⁴ together with the subsequent response to test doses reported by the latter, indicate a relation of urinary excretion to riboflavin nutrition similar to that which we have found for thiamin.^{27, 28}

In the case of nicotinic acid, there is no close correlation between the content of body fluids and that of tissues. Both Kohn³⁵ and Elvehjem³⁶ and their co-workers, using biologic methods of assay, have reported that the nicotinic acid-containing enzymes in the blood are not significantly diminished in experimental black tongue or in human pellagra, although the tissues are depleted. Using our chemical method³⁷ and trying various methods of "saturation" tests, we³⁸ have not found much promise for the direct laboratory diagnosis of nicotinic acid deficiency.

The significance of blood analyses of vitamin A remains to be determined. Wide variations in apparently normal people have been found by Lindqvist.⁵ McCoord and Luce-Clausen³⁹ have found no significant increase in the blood content of rats fed supplements of vitamin A, although the content of their livers was greatly increased over the controls. Lindqvist⁵ found no significant change in the blood values of human beings fed large supplements of vitamin A.

Vitamin A is insoluble in water. Josephs⁴⁰ has reported a rough correlation between the vitamin A and cholesterol and total lipids in the blood in persons whose nutritional status was not evaluated. This had been previously a subject of interest. Lindqvist⁴¹ reported that during convalescence from pneumonia serum vitamin A values rose before there was any change in blood cholesterol. It might be expected that there should be a closer correlation of the vitamin A of the serum with its neutral fats than with its lipids.

Another approach to the clinical laboratory diagnosis of vitamin deficiency is the measurement of resultant disturbances of body function. When the specificity for a vitamin deficiency of the disturbance of body function and its sensitivity are established, such a method may help to establish a normal level of vitamin nutrition. The question of specificity and sensitivity makes most of such methods of doubtful value.

Attempts have been made to diagnose vitamin C deficiency by measurement of capillary fragility. As in other studies, without chemical control, Abt, Farmer, and Epstein⁴² did not find any uniform relation between capillary fragility and blood ascorbic acid or dietary history. Considering the number of factors concerned in purpura and its inconstancy in scurvy, this is not surprising.

Pyruvic acid in blood and urine has been studied in relation to thiamin nutrition. Embden and associates⁴³ showed that pyruvic acid is a normal intermediary in the carbohydrate metabolism of muscle. Peters and Sinclair⁴⁴ identified pyruvic acid in lactate solutions in which avitaminose pigeon brain had been incubated. Thompson and Johnson⁴⁵ demonstrated an increase of pyruvic acid in the blood of avitaminose pigeons. Platt and Lu⁴⁶ have shown increases of pyruvic acid in the blood of all cases of fulminating beriberi but in only a portion of their subacute cases. They have proposed⁴⁷ the determination of the increase of blood pyruvate after exercise as a test for thiamin deficiency, but their reported results were inconsistent. They did not report the amount of exercise used and their test perhaps deserves further investigation.

Attempts have been made to replace the tedious hydrazone method of blood pyruvate analysis by determination of bisulfite-binding substances (B.B.S.) in the blood. Bisulfite-binding substances include other carbonyl compounds. While in experimental animals the blood bisulfite-binding substances have been found to be well correlated with thiamin deficiency, in man such correlation has been found neither with clinical evidence of thiamin deficiency⁴⁸ nor with our determinations of urinary thiamin.⁴⁹ Bauerji and Harris⁵⁰ have shown that the addition of sodium lactate to the diet caused increased excretion of bisulfite-binding substances in the urine of rats with vary-

ing grades of thiamin deficiency. They proposed a similar procedure for testing human beings, but stated that preliminary tests indicated that it may be necessary to modify the procedure in some details.

Night blindness has been known since the days of Hippocrates when one of the methods of its treatment was a liver diet. Block in 1921⁵¹ suggested that it was due to vitamin A deficiency. Fridericia and Holm⁵² showed that in experimental animals the concentration of visual purple in the retina was dependent upon vitamin A in the diet and that without it they became night blind.

Attempts to apply determination of dark adaptation to the evaluation of vitamin A nutrition have resulted in most conflicting reports. It seems probable that these discrepancies are due to varying techniques which may not adequately control the biochemical factors involved, and possibly to the influence of other vitamins upon dark adaptation. Harris and Abbasy⁵³ point out that most of the failures to correlate dark adaptation with vitamin A nutrition have been by those using the biophotometer. Possible inadequacies of the procedure used with this apparatus have been discussed by them, by Hecht and Mandelbaum,⁵⁴ and by Thomson and co-workers.⁵⁵ The extensive study of dark adaptation by Hecht⁵⁶ furnishes a basis for clinical application. Thomson and co-workers^{55a} reported that ten minutes of preliminary dark adaptation, followed by four minutes of bleaching with a bright light, was not adequate to eliminate the effect of previous light. Even twenty minutes of dark adaptation was not adequate for some subjects.^{55b} Harris and Abbasy⁵³ considered the bleaching light of the biophotometer too weak. Hecht⁵⁶ has shown the difference in the dark adaptation of cones and rods and the desirability of testing a known segment of the retina because of the different distribution of cones and rods. It will always be necessary to have calibrated accurately the intensity of the measuring light, and to eliminate the influence of unrelated ocular conditions. Nevertheless, the results which we have seen obtained by Curtis and his associates⁵⁷ indicate that useful studies can be done with the biophotometer—perhaps because these studies were on in-patients protected from strong light.

The suggestion of Hecht and Mandelbaum⁵⁴ that the slow recovery of dark adaptation in their patients receiving large supplements of vitamin A, compared with the very rapid recovery of dark adaptation in the patients of Wald, Jeghers, and Arminio⁵⁸ and others, was due to their failure to add supplements of other vitamins to their experimental diet is probably correct. The retinas of many species of animals have been found by von Euler and Adler⁵⁹ and others to contain relatively high concentrations of riboflavin which has been supposed to be concerned with dim vision. Sydenstricker and his associates⁶¹ reported that dimness of vision is a common symptom of riboflavin deficiency in human beings. Kimble and Gordon⁶⁰ reported that individuals who had failed to improve their dark adaptation with supplements of vitamin A were promptly restored when riboflavin was also added. In some others who failed to improve with riboflavin, ascorbic acid supplements were followed by satisfactory improvement. It is possible that some of the discrepancies in the reports concerning the relation of vitamin A and dark adaptation are due to failure to eliminate the influence of other vitamins.

The relation of abnormal pigments in urine to pellagra will become of especial importance if a method for direct determination of nicotinic acid nutrition is not devised. Beekh, Ellinger, and Spies⁶¹ reported the occurrence in the urine of pellagrins of large amounts of porphyrins. Later Spies and his associates⁶² called the pigment "porphyrin-like substances." They reported⁶³ that the pigment excretion returned to normal in from one to six days in 16 pellagrins receiving vitamin supplements. But in 4 pellagrins on a basal diet without supplements, the pigment excretion also returned to normal, although only after four to six weeks. Watson⁶⁴ and Meiklejohn and Kark⁶⁵ have identified the abnormal urinary pigment as uroscopin and probably another related pigment. They report the occurrence of these pigments in the urine of patients with diseases other than pellagra. The possibility of an associated vitamin deficiency in such patients and the relation of such pigment excretion to liver diseases requires further investigation.

REFERENCES

1. Field, H., Jr., Parnall, C., Jr., and Robinson, W. P.: *New England J. Med.* 223: 307, 1940.
2. Ecker, E. E., and Pillemmer, L.: *J. A. M. A.* 112: 1449, 1939.
3. Carr, F. H., and Price, E. A.: *Biochem. J.* 20: 497, 1926.
4. von Eckelen, M., and Emmerie, A.: *Acta brev. Neerland.* 4: 171, 1935.
5. Lindqvist, T.: *Acta med. Scandinav. Suppl.* 97, pp. 1-262, 1938.
6. Dann, W. J., and Evelyn, K. A.: *J. Biol. Chem.* 32: 1008, 1938.
7. Kimble, M. S.: *J. LAB. & CLIN. MED.* 24: 1055, 1939.
8. Zilva, S. S.: *Biochem. J.* 21: 689, 1927.
9. Tillmans, J., et al.: *Ztschr. f. Untersuch. d. Lebensmitt.* 60: 34, 1930; 63: 21, 24, 267, 1932.
10. Clark, W. M., et al.: *Hyg. Lab. Bull.* 151: 151, 1928; Gibbs, H. D., Cohen, B., and Caunan, R. K.: *Pub. Health Rep.* 40: 649, 1925.
11. Farmer, C. J., and Abt, A. F.: *Proc. Soc. Exper. Biol. & Med.* 32: 1625, 1935.
12. Pijoan, M., and Klemperer, F.: *J. Clin. Investigation* 16: 443, 1937.
13. Mindlin, R. L., and Butler, A. M.: *J. Biol. Chem.* 122: 673, 1938.
14. Evelyn, K. A., Malloy, H. T., and Rosen, C.: *J. Biol. Chem.* 126: 645, 1938.
15. Roe, J. H., and Hull, J. M.: *J. Biol. Chem.* 128: 329, 1939.
16. Bessey, O. A.: *J. Biol. Chem.* 126: 771, 1938.
17. Peters, R. A.: *Nature* 135: 107, 1935.
18. Jansen, B. C. P.: *Rec. trav. chim.* 55: 1046, 1936.
19. Goudsmit, J., and Westenbrink, H. G. K.: *Nature* 139: 1108, 1937.
20. Melnick, D., and Field, H., Jr.: *J. Biol. Chem.* 127: 505, 1939.
21. Melnick, D., and Field, H., Jr.: *J. Biol. Chem.* 127: 515, 531, 1939; *Ibid.* 130: 97, 1939.
22. Prebluda, H. J., and McCollum, E. V.: *Science* 84: 488, 1936; *J. Biol. Chem.* 127: 495, 1939.
23. Meiklejohn, A. P.: *Biochem. J.* 31: 1441, 1937.
24. Sinclair, H. M.: *Biochem. J.* 33: 2027, 1939.
25. Goodhart, R., and Sinclair, H. M.: *Biochem. J.* 33: 1099, 1939.
26. von Eckelen, M.: *Biochem. J.* 30: 229, 1936.
27. Robinson, W. D., Melnick, D., and Field, H., Jr.: *J. Clin. Investigation* 19: 399, 1940.
28. Melnick, D., Field, H., Jr., and Robinson, W. D.: *J. Nutrition* 18: 593, 1939.
29. Goodhart, R., and Sinclair, H. M.: *J. Biol. Chem.* 132: 11, 1940.
30. Sebrell, W. H., and Butler, R. E.: *Pub. Health Rep.* 53: 2282, 1938.
31. Sydenstricker, V. P., Sebrell, W. H., Cleckley, H. M., and Kruse, H. D.: *J. A. M. A.* 114: 2437, 1940.
32. Ferrebee, J. W.: *J. Clin. Investigation* 19: 251, 1940.
33. Vivaneo, F.: *Naturwissenschaften* 23: 306, 1935.
34. Emmerie, A.: *Acta brev. Neerland.* 7: 71, 1937.
35. Kohn, H. I., and Bernheim, F.: *J. Clin. Investigation* 18: 585, 1939; Kohn, H. I., and Klein, J. R.: *J. Biol. Chem.* 130: 1, 1939.
36. Axelrod, A. E., and Elvehjem, C. A.: *J. Biol. Chem.* 131: 77, 1939; Axelrod, A. E., Gordon, E. S., and Elvehjem, C. A.: *Am. J. M. Sc.* 199: 697, 1940.
37. Melnick, D., and Field, H., Jr.: *J. Biol. Chem.* 130: 97, 1939.
38. Robinson, W. D., Melnick, D., and Field, H., Jr.: Unpublished observations.
39. McCoord, A. B., and Luee-Clausen, E. M.: *J. Nutrition* 7: 557, 1934.
40. Josephs, H. W.: *Bull. Johns Hopkins Hosp.* 65: 112, 1939.

41. Lindqvist, T.: *Klin. Wehnschr.* 16: 1345, 1937.
42. Abt, A. F., Fnrmer, C. J., and Epstein, I. M.: *J. Pediat.* 8: 1, 1936.
43. Embden, G., Deuticke, H. J., and Kraft, G.: *Klin. Wehnschr.* 12: 343, 1933.
44. Peters, R. A., and Sinclair, H. M.: *Biochem. J.* 27: 1910, 1933.
45. Thompson, R. H. S., and Johnson, R. E.: *Biochem. J.* 29: 694, 1935.
46. Platt, B. S., and Lu, G. D.: *Biochem. J.* 33: 1525, 1939.
47. Lu, G. D., and Platt, B. S.: *Biochem. J.* 33: 1538, 1939.
48. Taylor, F. H. L., Weiss, S., and Wilkins, R. W.: *J. Clin. Investigation* 16: 833, 1937.
49. Robinson, W. D., Melnick, D., and Field, H., Jr.: *J. Clin. Investigation* 19: 483, 1940.
50. Banerji, G. G., and Harris, L. J.: *Biochem. J.* 33: 1346, 1939.
51. Block, C. E.: *J. Hyg.* 19: 283, 1921.
52. Fridericin, L. S., and Holm, E.: *Am. J. Physiol.* 73: 63, 1925; Holm, E.: *Am. J. Physiol.* 73: 79, 1925.
53. Harris, L. J., and Abbasy, M. H.: *Lancet* 2: 1299, 1939.
54. Hecht, S., and Mandelbaum, J.: *J. A. M. A.* 112: 1910, 1939.
55. (n) Thomson, A. M., Griffith, H. D., Mutch, J. R., and Lubbock, D. M.: *Brit. J. Ophthalm.* 23: 461, 1939.
- (b) Thomson, A. M., Griffith, H. D., Mutch, J. R., Lubbock, D. M., Owen, E. C., and Logaras, G.: *Ibid.* 23: 697, 1939.
56. Hecht, S.: *Physiol. Rev.* 17: 239, 1937.
57. Curtis, A. C., and Brunzer, J. G.: *Arch. Int. Med.* 65: 90, 1940.
58. Wald, G., Jeghers, H., Arminio, J.: *Am. J. Physiol.* 123: 732, 1938.
59. von Euler, H., and Adler, E.: *Ztschr. f. physiol. Chem.* 228: 1, 1934.
60. Kimble, M. S., and Gordon, E. S.: *J. Biol. Chem.* 128: 1939. *Proc. Lii.*
61. Beckh, W., Ellinger, P., and Spies, T. D.: *Quart. J. Med.* 6: 305, 1937.
62. Spies, T. D., Bean, W. B., and Ashe, W. F.: *Ann. Int. Med.* 12: 1839, 1939.
63. Spies, T. D., Sasaki, Y., and Gross, E. S.: *South. M. J.* 31: 483, 1938.
64. Watson, C. J.: *Proc. Soc. Exper. Biol. & Med.* 39: 514, 1938; *Ibid.* 41: 591, 1939.
65. Meiklejohn, A. P., and Kark, R.: *New England J. Med.* 221: 519, 1939.

THE ROLE OF BLOOD CLOTTING ANOMALIES IN THE HEMORRHAGIC DISEASES

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BLEEDING involves factors of positive causation as well as of failure to control. Causes include injuries and factors relating to capillary fragility. The latter is increased in scurvy and in capillary and thrombocytopenic purpuras. It has not been convincingly proved that the citrus juice factor which appears to control capillary resistance is the vitamin P of Szent-Györgyi and his colleagues,¹ vitamin C, or an unidentified agent. Vitamin C is ascorbic (cevitamic) acid,² whereas vitamin P or citrin consists of flavone-glucosides.³

Arrest of hemorrhage and plugging of the vascular leak involve vasoconstriction and thrombus formation. These are illustrated in some published experiments³ on frog mesenteric capillaries. Fig. 1* shows an endothelial cell selectively stimulated by micromanipulation. The ensuing contraction partially occludes the lumen of the capillary. As shown in Fig. 2,[†] a more vigorous pricking of the endothelium has caused injury to the vessel wall, but the escape of blood is prevented by the formation of a thrombus consisting, at first, solely of cells. If the blood flow is sufficiently slow, the red blood cells take part and, in the example cited, they form the whole mass, except for a single white cell (IV) seen clinging to the distal end of the clump. With a faster blood flow only the "stickiest" cells adhere and agglutinate. In the mammal these are the platelets, some of which are shown in the blood clot in Fig. 3. Note the little vesicles visible under the dark-field illumination. These represent the result of a peculiar form of lysis which occurs in platelets just prior to blood coagulation.⁴ The altered platelets provide some agent which takes part in blood coagulation, and a network of fibrin threads (a few of which are seen in Fig. 3) binds the cell thrombus more firmly together. The point to be emphasized, however, is that the special function of platelets and other cells in thrombus formation is to adhere together and add mechanical strength and tenacity to the clot. Platelets are believed also to aid in the clot retraction which makes for additional firmness. We shall make further reference to their role in blood coagulation.

The following deals principally with the clotting mechanism proper in an attempt to show how our current knowledge,⁵ largely derived from *in vitro* experiments, helps to rationalize many of the clinical problems of the so-called "hemorrhagic diathesis" or "bleeding tendency."

Fig. 4 presents a scheme of the essential clotting reactions. The main phenomena are well understood: a coagulant (thrombin) is first formed from an

*Reference 3, Fig. 2, p. 463.

†Reference 3, Fig. 4, p. 464.

inactive precursor (prothrombin) and then acts on fibrinogen to form fibrin. Prothrombin and fibrinogen are prepared by empirical methods from the globulin fraction of the plasma proteins. The conversion of prothrombin to thrombin requires certain activating agents. The first is ionized calcium salts.⁶ The second is the so-called thromboplastic factor which has come into new prominence in the last few years. The phospholipid cephalin has an important role, but there are undoubtedly other accessory factors (*vide infra*). The clotting reactions may be inhibited by specific agents, acting either upon the process of prothrombin activation (whence the designation "antiprothrombic") or upon



Fig. 1.—Untouched photomicrograph, showing localized contraction of single endothelial cell (E) of frog's mesenteric capillary, in response to micromanipulation by needle (N). The appearance prior to stimulation was very similar to that of an endothelial cell (en.). A previously manipulated Rouget cell (R) may be drawn slightly from the capillary wall without affecting the underlying blood vessel. There is "physiologic flow," unrelated to the manipulative procedures.



Fig. 2.—Untouched photomicrograph (frog mesentery), showing (1) maximal capillary dilation and rapid blood flow due to ineipient inflammatory reaction; (2) formation of red blood cell "thrombus" (T) at site of endothelial injury, effected by microdissection needle [a single white corpuscle (W) is attached to lower pole of thrombus]; (3) the constricting effect of connective tissue bands (B); (4) a marginating leucocyte (M); and another (E) emigrating through the wall of capillary (C).

the thrombin-fibrinogen interaction (hence the term "antithrombic"). The fundamental reactions are subject to a variety of nonspecific influences, such as temperature, pH, salt concentration, "wetting" (lowering of surface tension), dilution, and the like.

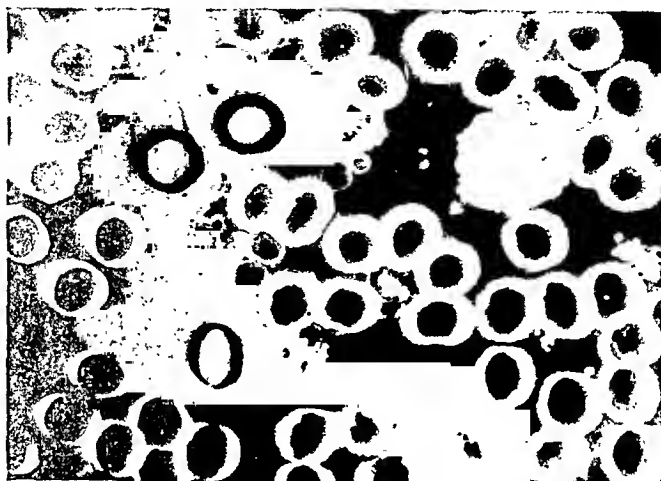


Fig. 3.—Dark-field photomicrograph of coagulated human blood drop. Note especially the vesicular formations from altered platelets and the "needles" of fibrin. The field also includes numerous refractile erythrocytes and a single polymorphonuclear (neutrophilic) leucocyte.

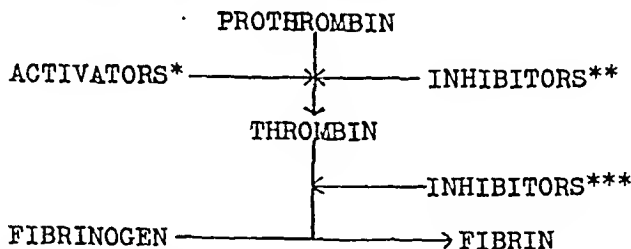


Fig. 4.—*Activators: (1) Calcium salts. (2) Thromboplastic agents, including (a) phospholipids (cephalin), (b) ? thromboplastic enzyme, and (c) ? diffusible factor.

**Inhibitors: Antiprothrombins, including heparin and other sulfonic substances.

***Inhibitors: Antithrombins, including hirudin, heparin plus plasma-albumin, etc.

Nonspecific factors: Temperature, pH, salt concentration, "wetting," etc.

Fig. 5 shows the chief steps in a typical experiment to analyze the clotting mechanism by purifying its components with modern methods.⁷ The diagram illustrates the clotting time on recalcifying (with and without added cephalin) the various fibrinogen-containing materials. Each interval represents one minute. An especially careful technique in collection of the blood, repeated centrifugalization, and Berkefeld filtration of the plasma insure a plasma quite free from any products of tissue, platelet, or other cellular (including bacterial) origin. Such plasma invariably clots in about ten minutes or less on simple recalcification (No. 4). This is a minor delay over the two to four minutes for unfiltered plasma, but the clotting time is easily restored to normal by adding a trace of pure cephalin (No. 5). The important conclusion is that the blood plasma itself contains all the essential factors for clotting, including much more phospholipid than the whole of the platelet content. Of course, maintenance of the normal fluidity of blood must mean that these factors are not all "avail-

able." It is quite possible that the thromboplastic factor in the platelets is more readily available than that of the plasma, but we are inclined to minimize the role of platelets and similar material in blood clotting. Hence, we do not attribute the bleeding in cases of thrombocytopenia to any significant clotting anomaly, but refer to other explanations, including (1) decreased capillary resistance to injury, and (2) poor thrombus formation in terms of mechanical properties, such as adhesiveness and retractibility. These have already been discussed. Clotting time is normal in thrombocytopenic purpura; it is only the bleeding time which is prolonged.

CLOTTING PROPERTIES OF REAGENTS PREPARED FROM DOG BLOOD

Protocol. Temperature : 38°C. pH : 7.5

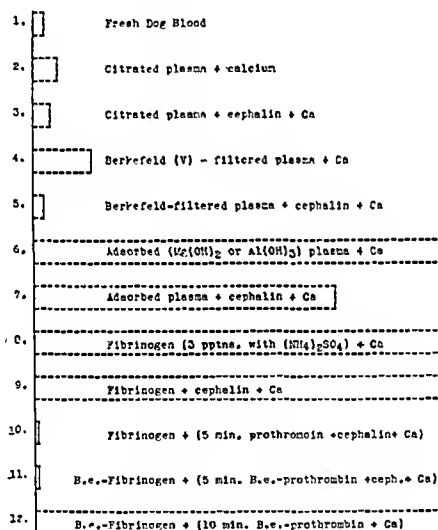


Fig. 5.

Returning to the fibrinogen, it is seen that the final preparations (Nos. 8, 9) show no clot on recalcification even with the addition of cephalin or other thromboplastic agents. This means that we have eliminated all traces of prothrombin. Clotting occurs, however, in less than one minute on the addition of an active thrombin preparation. We have recently succeeded in obtaining fibrinogen in the dry state by means of the lyophilic technique.⁸

Fibrinogen, of course, is the sine qua non of the fibrin clot. It is found that the fibrinogen concentration in experimental tests may be varied widely with only a minor influence on coagulation time. The character of the clot alters. Below a certain concentration, the coagulum formed is very weak,

suggesting that the equivalent *in vivo* would not be much good for the arrest of hemorrhage. There are marked variations in the blood fibrinogen content in various diseases, and hemorrhagic conditions associated with lowering of the plasma fibrinogen are clearly recognized although they appear to be rare. Pseudohemophilia is said to fall into this class. The typical case is familial and has a long bleeding time, but a normal platelet count.⁹ A simple plasma protein analysis would establish the fibrinogen deficiency but, as far as we are aware, this has seldom been reported in the clinical literature. In the fibrinogen deficiencies accompanying liver dysfunction, the prothrombin is usually lowered as well and this often precedes the fibrinogen decrease. Naturally, one would not expect to benefit these patients with any measures aimed solely at increasing the thrombic function. The rational treatment is to tide over the hemorrhagic crisis with transfusions and subsequently to build up the blood proteins by dietary and other means.

RATE OF THROMBIN FORMATION: PROTHROMBIN (10) + $\frac{N}{10}$ CaCl₂ (1)
+ 0.1% CEPHALIN (1). DOG. 38°C.

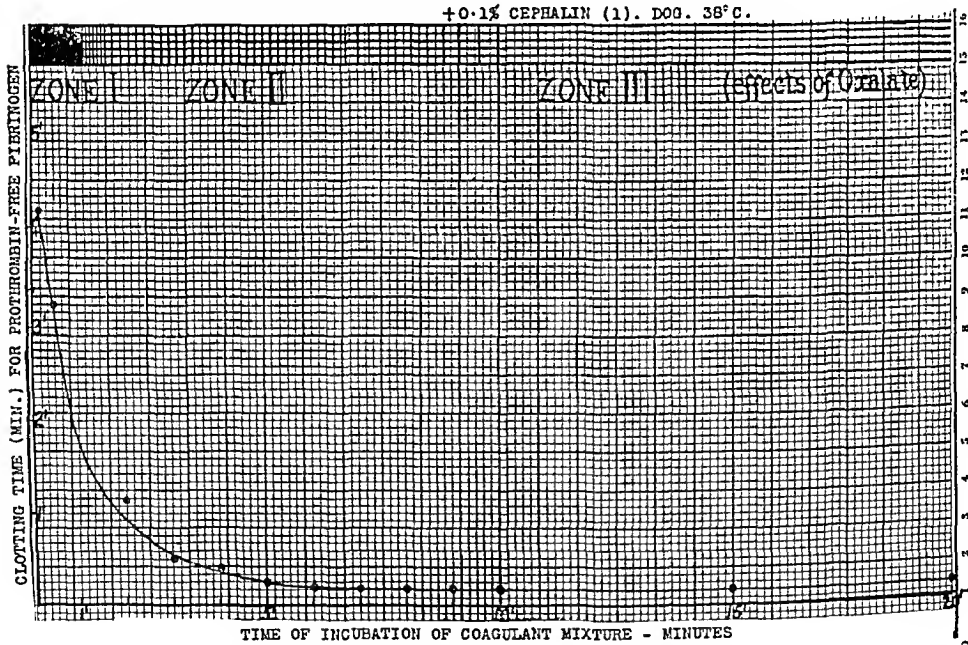


Fig. 6.—Prothrombin activation curve. This affords information as to the rate and amount of thrombin formation. The role of calcium is evident from the different effects of oxalate (or citrate) at the three zones depicted. In Zone I thrombin formation is completely inhibited; in Zone II the oxalate progressively removes calcium and inactivates the "intermediary" complex; in Zone III the "ripe" thrombin resists the decalcifying action of oxalate, etc.

Most of our recent work concerns the process of conversion of prothrombin to thrombin. We have little to say as to the nature of prothrombin. It is obtained from plasma by various precipitation methods.⁷ The best preparations contain proteins, phospholipids, salts, etc. However, it is significant that the agents necessary to activate prothrombin are being more and more clearly defined. Prothrombin, in our experience, is never activated spontaneously. It needs calcium salts and usually some thromboplastic agent in addition. In the last two experiments of Fig. 5, the prothrombin and fibrinogen solutions were

extracted with benzene prior to the tests.¹⁷ The emphasis is on the result rather than the interpretation of the technique. Experiment No. 11 shows that the benzene-extracted fibrinogen is readily clotted by a thrombin made from the benzene-extracted prothrombin by five minutes' incubation with calcium and cephalin. Experiment No. 12 shows that calcium alone is insufficient. We shall return to this point later.

Fig. 6 illustrates the standard technique with which we follow the process of thrombin formation. A mixture of prothrombin and calcium salt plus any activator or inhibitor we wish to investigate is incubated, preferably at a cool temperature (10 to 20° C.), in order to control possible deterioration.⁷ At various time intervals, charted on the abscissa of the graph, a measured sample is removed and added to a fixed amount of fibrinogen (under constant conditions), and the clotting time is noted and plotted on the ordinate of the graph. Clotting time is a measure of thrombin concentration and gives us a relative value for the amount of thrombin present at the time the test is made. The shorter the clotting time, or the lower the curve, the more potent is the thrombin. We may term the graph the "activation curve" of the particular thrombin mixture. We have published¹⁰ a series of such curves with varying amounts of cephalin. The more phospholipid, the lower the curve, i.e., the greater the thrombin formation. The same sort of thing occurs, but even more strikingly, when we vary the calcium.¹⁰

TABLE I
SCHEMA OF THROMBIN FORMATION

Plasma globulins (excluding fibrinogen) or agent associated with proteins	1. Plasma lipoproteins 2. Platelets 3. Other cells and tissues	Blood calcium	
Prothrombin (? protein)	"Available" cephalin (phospholipid)	Calcium salts (ionized)	Zone I
	Intermediary complex (protein-phospholipid-calcium)		Zone II
	"Ripe" thrombin		Zone III

Table I summarizes our view as to the fundamental reactions of thrombin formation. Evidence previously published¹¹ supports the view that there is the formation of an intermediary colloidal complex containing prothrombin, cephalin, and calcium in definite quantitative proportions. The final, older ("ripe") thrombin may be prepared free from all traces of calcium; it retains considerable activity after alcohol-ether (3:1) extraction (at room temperature) and is well preserved under acetone.

It is only the ionized form of calcium which takes part in the conversion of prothrombin to thrombin.¹² Ransmeier and McLean¹³ have recently found the optimum calcium ion concentration for plasma clotting to be almost identical with the normal value for "diffusible" calcium in serum. Too much calcium

is inhibitory, a nonspecific salt (cation) effect.¹⁴ Too little fails to convert all the prothrombin. The minimum calcium ion concentration for clotting is below the lowest serum calcium level compatible with life, at least in experimental dogs. It would seem unlikely that clinical variations in the serum calcium level can materially affect clotting time or that calcium administration can be expected to exert any therapeutic benefit attributable to assistance in the clotting mechanism.

Typical prothrombin preparations are very poorly activated by calcium alone, and some solutions (Fig. 5) are quite unable to clot fibrinogen on simple recalcification. In this case a so-called "thromboplastic agent" is an essential addition. The phospholipid cephalin, as isolated from brain and other tissue sources, is such a thromboplastic agent. Crude aqueous tissue extracts, known as thrombokinas or thromboplastins, act similarly,¹⁵ but we shall later point out some significant differences. Pure cephalin is surprisingly active, a solution as great as 1:1,000,000 exerting a definite thromboplastic effect. Some snake venoms are more than one hundred times more active than this.¹⁶ We have analyzed blood plasma, platelets, and corpuscles from the various hemorrhagic diseases without finding any significant decrease in total phospholipid or cephalin content.¹⁷

Prothrombin solutions, which were very poorly activated by calcium alone, analyzed some 8 to 30 mg. per cent of total phospholipid, of which about 40 to 60 per cent was cephalin (choline-free). These amounts of cephalin, added in the form of a solution of the isolated phospholipid, produce rapid and abundant thrombin formation. There is an experimental difference, therefore, between cephalin in simple solution and the form in which it occurs in the natural materials. We now have clear evidence¹⁷ that the cephalin in practically every crude material studied (and this applies to plasma, prothrombin, platelets, lung and brain extracts) is all in firm combination with the proteins present. This is true, at least, of the ability to extract it with simple fat solvents, such as ether, benzene, and the like. With regard to the crude thromboplastins (or aqueous tissue extracts), it has become evident in contemporary methods of prothrombin estimation¹⁹ that these materials are much more active than the equivalent of their isolated phospholipids. Our quantitative data prove this beyond a reasonable doubt. We are thus led to two conclusions, viz., (1) that the phospholipid of the natural thromboplastic system is not necessarily "available" for clotting, and (2) that a new factor must be sought for to explain the full mechanism of thromboplastic action. The latter conclusion is foreshadowed in the investigations of plasma or serum globulin in hemophilia by Bendien and van Creveld,²⁰ of Amsterdam, and also by a group of Harvard workers.²¹ Howell, of Baltimore, has reported preliminary attempts at isolation, but his published data²² neither suggest the possibility of an enzyme nor do they suffice to rule it out. In deference to these and other claims,²³ we have included a possible diffusible factor among accessory thromboplastic agents (Fig. 4).

An extensive series of comparative experiments with Kunitz and Northrop's crystalline trypsin leads us to believe that such an enzyme may be the

thromboplastic factor for which we are looking. The published data¹⁷ may be condensed into the statement that an artificial system of trypsin plus calcium closely resembles aqueous tissue extracts in every reaction tested. Cephalin is also adjuvant, and, in such experimental differences between cephalin and crude thromboplastin as can be defined, the new mixture provides a means of bridging the divergence of findings. The accessory thromboplastic action of crystalline trypsin is evident in the thrombin formation data of Table II.

TABLE II

THE ROLE OF CALCIUM, CEPHALIN, AND CRYSTALLINE TRYPSIN IN EXPERIMENTAL THROMBIN FORMATION

Thrombic mixtures (T) = 4.0 c.c. prothrombin solution + 1.0 c.c. activating agents (suitably diluted). Calcium is expressed in milliequivalents, and trypsin and cephalin in milligrams, per 5.0 c.c. of thrombic mixture. Thrombin formation at 15° C. Clotting times for 0.5 c.c. T + 1.0 c.c. fibrinogen solution (prothrombin-free) at 38° C.

T	ACTIVATING AGENTS IN THROMBIC MIXTURE	THROMBIN INCUBATION PERIOD (MINUTES) AT 15° C.							
		2'	5'	10'	20'	30'	45'	60'	180'
1	Calcium (0.025)		203"	167"	55"	28"	19"	13"	47"
2	Calcium (0.025) + cephalin (0.25)		47"	35"	25"	22"	19"	18"	18"
3	Calcium (0.025) + cephalin (0.25) + trypsin (0.05)	40"	27"	20"	20"	20"		20"	
4	Calcium (0.025) + trypsin (0.05)		180"	145"	25"	20"		20"	
5	Trypsin (0.05)				120"	40"		25"	

Tryptic activity in blood plasma and tissue extracts has been previously established and is a complex story, involving an inactive enzyme precursor which must be freed from a specific inhibitor and then activated by a special Kiasase.²⁴ We are currently engaged in the task which has been opened up for correlating all this with blood coagulation. In the meantime, it can be stated that the ability of both trypsin and tissue extracts to restore the coagulability of hemophilic blood *in vitro* is due to the acceleration of the conversion of prothrombin to thrombin, delay in which has long been recognized to underlie the coagulation defect of hemophilia.¹⁸ This is shown for trypsin in Table III;⁶ tissue extracts are similar; cephalin has little or no influence. There does not seem to be anything wrong with the tissue factor in hemophilia, which probably accounts for the normal bleeding time which is usually obtained by the Duke test in which, of course, the blood flows over the tissues damaged by the puncture needle. There is an unusually slow breakdown of platelets in hemophilia,⁵ but it is difficult to say whether this is the cause or the effect of the plasma deficiency. Our suggestion is that hemophilia is a deficiency in the plasma content of the new thromboplastic factor, which we are tentatively calling "thromboplastic enzyme." We have reason to believe that the enzyme normally acts by "mobilizing" calcium and cephalin for the conversion of prothrombin to thrombin.¹⁷

Prothrombin is the protein component of the clotting agent. We are not prepared to discuss the hypothesis advanced by Eagle,²⁵ which suggests that it assumes an enzyme-character after conversion to thrombin, but we would like to review current methods of prothrombin estimation and the role of vitamin

*Reference 18, Table II.

K in the treatment of prothrombin deficiencies. Clotting time in fibrinogen-thrombin mixtures is a linear function of the thrombin concentration. If, therefore, adequate calcium and thromboplastin are added to convert all prothrombin to stable thrombin, it is possible to obtain a measure of prothrombin concentration in plasma, etc., in terms of an empirical standard control.

TABLE III

ACTIVATION OF RECALCIFIED HEMOPHILIC "GLOBULIN SUBSTANCE" (PROTHROMBIN) BY CRYSTALLINE TRYPSIN

G_1 = 2.0 c.c. hemophilic "globulin substance" (G) + 0.4 c.c. distilled water + 1.6 c.c. N/10 CaCl_2 .

G_2 = 2.0 c.c. hemophilic "globulin substance" (G) + 0.4 c.c. (= 0.1 mg.) trypsin + 1.6 c.c. N/10 CaCl_2 .

Mixtures held at 24° C.; 0.25 c.c. test portions added to 0.5 c.c. quantities of hemophilic citrated plasma (same case) at times shown. Clotting times, seconds, at 38° C.

	THROMBIN FORMATION PERIOD, MINUTES, AT 24° C.					
	1'	5'	10'	20'	30'	45'
G_1	270"	180"	145"	135"	105"	62"
G_2	40"	17"	10"	7"	7"	7"

Controls.—1. 0.5 c.c. hemophilic plasma + 0.3 c.c. distilled water + 0.2 c.c. N/10 CaCl_2 = 720" (C.T.)

2. 0.5 c.c. hemophilic plasma + 0.1 c.c. H_2O + 0.2 c.c. "G" + 0.2 c.c. N/10 CaCl_2 = 275"

3. 0.5 c.c. hemophilic plasma + 0.3 c.c. H_2O + 0.2 c.c. (= 0.1 mg.) trypsin = no clot

4. 0.5 c.c. hemophilic plasma + 0.2 c.c. "G" + 0.2 c.c. CaCl_2 + 0.2 c.c. trypsin = 70"

There are many technical difficulties, the least controlled being the possibility of prothrombin deterioration. Quick's test²⁶ is a relatively simple attempt, consisting of oxalated plasma plus calcium plus brain thromboplastin, the clotting time of which is compared under standardized conditions with a normal control (or series). However, the test is really timing two independent reactions, viz., the conversion of prothrombin to thrombin on the one hand, and the actual fibrinogen-thrombin interaction on the other.²⁷ This is an important theoretical objection but may not be unduly significant for clinical interpretations. Smith and his colleagues²⁸ at Iowa have a more complicated technique which endeavors to separate the two phases of clotting. Without entering into a technical discussion, it may be stated, for the consideration of those who feel critically towards the new prothrombin tests, that they are still in a highly empirical stage. There is a tendency to avoid arbitrary "percentages" of prothrombin and to be satisfied with a comparison of the actual clotting times of the unknown and normal controls. As rough indices of plasma prothrombin content, these tests do give valuable information and there can be no doubt that prothrombin deficiency is an important factor in certain hemorrhagic conditions, such as vitamin K deficiency in chicks, certain toxic states in domestic animals, experimental and clinical disorders of the liver and biliary system, and, of special interest to pediatricians, hemorrhagic disease of the newborn.²⁹ All these are benefited by vitamin K preparations. This fat-soluble dietary factor³⁰ discovered by Dam (Copenhagen) and by Almquist (California) has now been shown to be a common property of a number of 1, 4-naphthoquinones, and we may shortly expect commercial exploitation of active synthetic products, as well as the natural concentrates of alfalfa, kale, spinach, tomatoes, dried

carrot tops, chestnut leaves, oak sprouts, soybean oil, putrefied fish meal, etc. Naphthoquinones are associated with bacterial pigment metabolism (as in the phthiocol from the tubercle bacillus). Alimentary tract microorganisms are thought to elaborate adequate vitamin K in most animals, with the possible exception of very young human infants. Bile is probably necessary for absorption of the vitamin. Dr. A. M. Snell³⁷ and his colleagues (Mayo Clinic) emphasize the importance of intestinal conditions in vitamin K absorption, and it must also be stressed that the effectiveness of therapeutic use is dependent upon an adequate liver function. Use in the hemorrhagic diseases is indicated in, and should be confined to, cases with prothrombin deficiency.

TABLE IV

EFFECTS OF HEPARIN ON THE FORMATION OF THROMBIN FROM RECALCIFIED PROTHROMBIN IN THE PRESENCE OF VARIOUS THROMBOPLASTIC AGENTS

Thrombic mixture (T) = 4 c.c. prothrombin solution + 1 c.c. activator (s) plus inhibitor (suitably diluted), incubated at 15° C. All mixtures include CaCl₂ (0.05 meq. Ca per 5 c.c. T). Cephalin, trypsin, and heparin : mg. per 5 c.c. T. Clotting Times (sec.) at 38° C. : 1 c.c. fibrinogen (prothrombin-free) + 0.5 c.c. T.

T	THROMBOPLASTIC AGENT	INHIBITOR	THROMBIN FORMATION PERIOD, MINUTES, AT 15° C.					
			1'	3'	10'	20'	30'	60'
1	*Cephalin (0.1)	—	—	50"	20"	11"	9"	9"
2	Cephalin (0.1)	Heparin (0.5)	—	∞	∞	∞	∞	∞
3	†Brain P-lipids (0.025)	—	50"	20"	10"	8"	6"	6"
4	Brain P-lipids (0.025)	Heparin (0.5)	∞	∞	∞	∞	1800"	360"
5	Thromboplastin (0.25 c.c.) including 0.025 mg. P-lipid	—	35"	16"	9"	7"	7"	7"
6	Thromboplastin (0.25 c.c.) including 0.025 mg. P-lipid	Heparin (0.5)	1720"	16"	10"	7"	7"	7"
7	Trypsin (0.125)	—	∞	30"	15"	12"	11"	8"
8	Trypsin (0.125)	Heparin (0.5)	∞	97"	32"	13"	11"	9"
9	Trypsin (0.05)	—	∞	95"	30"	15"	14"	11"
10	Trypsin (0.05)	Heparin (0.5)	∞	∞	660"	70"	32"	18"

*The cephalin was an old preparation which appears to have lost somewhat more than three-fourths of its original potency.

†Quick's "thromboplastin" extracted with alcohol-ether (3:1); acetone-insoluble P-lipids recovered in ether-petroleum ether, dried, and prepared in aqueous solution (1:10,000).

‡Indicates incomplete coagulation.

Bleeding in allergic states is no doubt a greater problem than can be explained in the light of enrrtent knowledge, but the following experiment from the Toronto laboratories³¹ appears to be of considerable significance. Dogs were thrown into anaphylactic shock after sensitization to alum-treated horse serum. During shock the blood became incoagulable and this was shown to be associated with a great outpouring of heparin. It could be demonstrated in the blood in several ways, including the actual isolation of the barium salt in crystalline form.³⁷ Heparin³² is a product originally isolated from the liver by Howell and Holt³³ in 1918, but now prepared from lung³⁴ in the Connaught laboratories at Toronto. It results from the combination of several organic acids, including mucicoin-polysulfuric acids, with the base glucosamine (Jorpes).³⁵

If the anaphylactic experiment is repeated after extirpation of the liver in an acute experiment, the dog still shows symptoms of shock, but the blood

is no longer incoagulable and there is no demonstrable heparin increase. It may be concluded, therefore, that in cases with demonstrable increase in the antithrombic factors in the blood, bleeding may be associated with the outpouring of heparin from liver reserves.

TABLE V

SYNERGISM BETWEEN ANTITHROMBIC ACTIONS OF HEPARIN (CONNAUGHT LABORATORIES) AND CRUDE PLASMA "ALBUMIN" (DIALYZED)

Thrombin: Stock preparation (prothrombin + calcium + cephalin + brain extract) kept under acetone and prepared for use by evaporating acetone and redissolving it in 0.9 per cent NaCl at pH 7.25.

	THROMBIN (C.C.)	DISTILLED WATER (C.C.)	HEPARIN (MG. IN 0.25 C.C.)	"ALBUMIN" (C.C.)	FIBRINOGEN* (C.C.)	CLOTTING TIME (38° C.)
1	0.5	0.5	-	-	1	14 sec.
2	0.5	0.25	-	0.25	1	18 sec.
3*	0.5	0.25	-	0.25	1	33 sec.
4	0.5	0.25	0.05	-	1	23 sec.
5	0.5	-	0.05	0.25	1	>4 hr.
6†	0.5	0.25	0.05	0.25†	1	26 sec.

*Fibrinogen added immediately, except in No. 3 where the addition was deferred for one hour; the increase in clotting time is scarcely significant in comparison with the immediate antithrombic effect noted in No. 5.

†A weak solution of dialyzed crystalline serum albumin (McMeekin) was used in No. 6.

As to the manner in which heparin acts, Howell and Holt (1918)³³ postulated a dual role. The first or "antiprothrombic" action (so-called) is the prevention of conversion of prothrombin to thrombin. This has recently been denied by at least four groups of workers (Mellanby, Quick, Smith and co-workers, and Astrup), but the last two admit that it can occur in the presence of an accessory plasma factor, possibly identical with the second mechanism (vide infra). We have³⁶ pointed out that all these recent workers used strong aqueous thromboplastins in converting the prothrombin to thrombin. If, however, only the isolated phospholipid is used, the classical antiprothrombic action of Howell is confirmed, without any accessory factor (Table IV).^{*} The inhibitory action, therefore, refers to the thromboplastic system rather than to the prothrombin itself. There is no doubt as to the second mode of action ("proantithrombic" of Howell and Holt) in which inactivation of fully formed thrombin is secured if, and only if, an additional factor, believed to be associated with plasma albumin (Quick) is present (Table V). We have recently performed some preliminary experiments with a pure crystalline serum albumin kindly supplied by Dr. T. L. McMeekin (Harvard) which accords with the findings of the Toronto workers³⁷ in failing to show definite antithrombic action with mixtures of crystalline albumin and heparin. This difference from the crude "albumin" is unexplained.

The real nature and mode of action¹⁴ of "antithrombin(s)" are not fully understood, but much significance attaches to the data of Fischer (Copenhagen)³⁸ showing the ability of heparin to form molecular compounds with proteins and protein split-products to make substances having a shift in their

*Reference 36, Table II.

isoelectric point in the acid direction. In situations where antithrombic factors are definitely demonstrable there can be little doubt as to their effectiveness in retarding in vivo as well as in vitro blood clotting.

SUMMARY

Prolongation of clotting time may contribute to those hemorrhagic states in which we can demonstrate (or postulate) either prothrombin or thromboplastin deficiencies or an excess of antithrombic factors. Calcium may be excluded from practical consideration. Factors such as fibrinogen and platelet defects play a role in determining the effectiveness of clotting and thrombosis in the arrest of hemorrhage. All these are controlling mechanisms, and the prior causative factors, including capillary injury, must also receive the fullest consideration.

REFERENCES

1. Armentano, L., Bentsath, A., Beres, T., Ruzsnyak, S., and Szent-Györgyi, A.: Über den Einfluss von Substanzen der Flavongruppe auf die Permeabilität der Kapillaren. Vitamin P, Deutsche med. Wochenschr. 62: 1325, 1936.
2. Szent-Györgyi, A.: Methoden zur Herstellung von Citrin, Ztschr. f. physiol. Chem. 255: 126, 1938.
3. See also Notes, Nature (London) 138: 27, 798, 1935, 1936.
4. King, C. G.: The Chemistry of Vitamin C, J. A. M. A. 111: 1462, 1938.
5. Ferguson, J. H.: Observations on Living Mesenteric Capillaries, J. LAB. & CLIN. MED. 22: 462, 1937.
6. Ferguson, J. H.: Observations on the Alterations of Blood Platelets as a Factor in Coagulation of the Blood, Am. J. Physiol. 108: 670, 1934.
7. Ferguson, J. H.: Blood: Coagulation, Biophysical Characters and Formed Elements, Ann. Rev. Physiol. 2: 71, 1940.
8. Ferguson, J. H.: Blood Calcium and the Calcium Factor in Blood Coagulation, Physiol. Rev. 16: 640, 1936.
9. Ferguson, J. H.: A Standardized Procedure for the Study of Coagulation Reactions (in vitro), J. LAB. & CLIN. MED. 24: 273, 1938.
10. Ferguson, J. H., and Erickson, B. N.: A New Fibrinogen Preparation, Proc. Soc. Exper. Biol. & Med. 40: 425, 1939.
11. Fowler, W. M.: Hereditary Pseudo-hemophilia, Am. J. M. Sc. 193: 191, 1937.
12. Ferguson, J. H.: Quantitative Relationships of Calcium and Cephalin in Experimental Thrombin Formation, Am. J. Physiol. 123: 341, 1938.
13. Ferguson, J. H.: An Intermediary Calcium Complex in Blood Coagulation, Am. J. Physiol. 119: 755, 1937.
14. Lebel, H., Schönheyder, F., and Muus, J.: The Relationship Between Coagulation Time of Plasma and the Concentration of Ionized and Unionized Calcium, Skandinav. Arch. f. Physiol. 78: 179, 1938.
15. Ransmeier, J. C., and McLean, F. C.: The Relationship of Calcium Ion Concentration to the Coagulation of Citrated Plasma, Am. J. Physiol. 121: 488, 1938.
16. Glazko, A. J., and Greenberg, D. M.: The Mechanism of the Inhibitory Effects of Electrolytes and Heparin on Blood Coagulation, Am. J. Physiol. 128: 399, 1940.
17. Ferguson, J. H.: An Experimental Analysis of Coagulant Activation, Am. J. Physiol. 117: 587, 1936.
18. Barratt, J. O. W.: Estimation of Fibrinogen and Thrombin, J. Physiol. 80: 422, 1934.
19. Ferguson, J. H., and Erickson, B. N.: The Coagulant Action of Crystalline Trypsin, Cephalin, and Lung Extracts, Am. J. Physiol. 126: 661, 1939.
20. Ferguson, J. H.: The Clotting of Hemophilic Plasma by Thromboplastic Enzyme, Am. J. Physiol. 126: 669, 1939.
21. Quick, A. J.: On Various Properties of Thromboplastin (Aqueous Tissue Extract), Am. J. Physiol. 114: 282, 1936.
22. Bendien, W. M., and van Creveld, S.: Investigations on Hemophilia, Am. J. Dis. Child. 54: 713, 1937; On Some Factors of Blood Coagulation, Especially With Regard to the Problem of Hemophilia, Acta med. Scandinav. 99: 12, 1939. Also see: Investigations on Hemophilia (I-IV), Acta brev. Neerland. 5: 135, 1935; 7: Nos. 1, 6, 7, 1937; 8: 136, 1938.
23. Patek, A. J., Jr., and Stetson, R. P.: Hemophilia. I. The Abnormal Coagulation of the Blood and Its Relation to the Blood Platelets, J. Clin. Investigation 15: 531, 1936.

- Patek, A. J., Jr., and Taylor, F. H. L.: Hemophilia. II. Some Properties of a Substance Obtained From Normal Human Plasma Effective in Accelerating the Coagulation of Hemophilic Blood, *Ibid.* 16: 113, 1937.
- Pohlé, F. J., and Taylor, F. H. L.: The Coagulation Defect in Hemophilia. The Effect in Hemophilia of Intramuscular Administration of a Globulin Substance Derived From Normal Human Plasma, *Ibid.* 16: 741, 1937; The Use of a Globulin Substance Derived From Beef Plasma as a Local Hemostatic in Hemophilia, *Ibid.* 17: 677, 1938; The Coagulation Defect in Hemophilia; Studies on the Refractory Phase Following Repeated Injections of Globulin Substance Derived From Normal Human Plasma in Hemophilia, *Ibid.* 17: 779, 1938.
- Lozner, E. L., Kark, R., and Taylor, F. H. L.: The Coagulation Defect in Hemophilia. The Clot Promoting Activity in Hemophilia of Berkefelded Normal Human Plasma Free From Fibrinogen and Prothrombin, *Ibid.* 18: 603, 1939.
- Lozner, E. L., and Taylor, F. H. L.: The Coagulation Defect in Hemophilia. Studies of the Clot Promoting Activity Associated With Plasma Euglobulin in Hemophilia, *Ibid.* 17: 821, 1939.
22. Howell, W. H.: Hemophilia (Wesley M. Carpenter Lecture), *Bull. New York Acad. Med.*, 2nd series 15: 3, 1939.
23. Larson, C. E., and Greenberg, D. M.: Evidence for the Presence of a Diffusible Organic Substance in Blood Which Accelerates Blood Clotting, *Proc. Soc. Exper. Biol. & Med.* 33: 305, 1935.
- Glazko, A. J., and Greenberg, D. M.: The Diffusible Organic Blood Clotting Factor, *Ibid.* 42: 177, 1939.
24. Schmitz, A.: Über die Proteinase des Fibrins (I), *Ztschr. f. physiol. Chem.* 244: 89, 1936; Über die Freilegung von aktivem Trypsin aus Blutplasma (II), *Ibid.* 250: 37, 1937; Über die Trypsin-Inhibitor des Blutes (III), *Ibid.* 255: 234, 1938.
25. Eagle, H.: Recent Advances in the Blood Coagulation Problem, *Medicine* 16: 95, 1937.
26. Quick, A. J.: The Nature of the Bleeding in Jaundice, *J. A. M. A.* 110: 1658, 1938. (Correction: *Ibid.* 111: 1775, 1938.)
27. Warner, E. D., Brinkhous, K. M., and Smith, H. P.: The Prothrombin Conversion Rate in Various Species, *Proc. Soc. Exper. Biol. & Med.* 40: 197, 1939.
28. Scanlon, G. H., Brinkhous, K. M., Warner, E. D., Smith, H. P., and Flynn, J. E.: Plasma Prothrombin and the Bleeding Tendency, *J. A. M. A.* 112: 1898, 1939.
- Smith, H. P., Ziffren, S. E., Owen, C. A., and Hoffman, G. R.: Clinical and Experimental Studies on Vitamin K, *J. A. M. A.* 113: 380, 1939.
29. Waddell, W. W., Jr., and Guerry, duP., III: Effect of Vitamin K on the Clotting Time of the Prothrombin and the Blood, With Special Reference to Unnatural Bleeding of Newly Born, *J. A. M. A.* 112: 2259, 1939.
- Owen, C. A., Hoffman, G. R., Ziffren, S. E., and Smith, H. P.: Blood Coagulation During Infancy, *Proc. Soc. Exper. Biol. & Med.* 41: 181, 1939.
- Quick, A. J., and Grossman, A. M.: *Ibid.* 41: 227, 1939.
30. Snell, A. M.: Vitamin K: Its Properties, Distribution and Clinical Importance, *J. A. M. A.* 112: 1457, 1939.
31. Waters, E. T., Markowitz, J., and Jaques, L. B.: Anaphylaxis in the Liverless Dog, and Observations on the Anticoagulant of Anaphylactic Shock, *Science* 87: 582, 1938.
32. Mason, M. F.: Heparin: A Review of Its History, Chemistry, Physiology and Clinical Applications, *Surgery* 5: 451, 618, 1939.
33. Howell, W. H., and Holt, E.: Two New Factors in Blood Coagulation—Heparin and Pro-antithrombin, *Am. J. Physiol.* 47: 328, 1918.
34. Charles, A. F., and Scott, D. A.: IV. The Preparation of Heparin From Beef Lung, *Tr. Roy. Soc. Canada*, 3rd ser., sect. V 28: 55, 1934.
35. Jorpes, E., and Bergström, S.: On the Relationship Between the Sulfur Content and the Anticoagulant Activity of Heparin Preparations, *Biochem. J.* 33: 47, 1939.
36. Ferguson, J. H.: Heparin and Plasma Albumin in Relation to Thromboplastic Action of Trypsin, Cephalin and Brain Extracts, *Proc. Soc. Exper. Biol. & Med.* 42: 33, 1939.
37. Proceedings of the American Physiological Society (including Symposium on Blood Coagulation), New Orleans, March, 1940.
38. Fischer, A.: Studies on Coagulation of the Blood, *Jap. J. Exper. Med.* 13: 223, 1935. (An excellent résumé, in English, of Fischer's extensive data.)

HEMOLYTIC ANEMIA*

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EVERY anemia results from a loss of balance between red blood cell formation and red blood cell destruction. A hemolytic anemia is one due to a more rapid destruction of erythrocytes than the bone marrow can replace. The normal method of disposal of worn-out, or otherwise injured, cells is by hemolysis. Almost a trillion, or one twenty-fifth, of all the circulating erythrocytes are so disposed of each day. Normally a delicate balance is maintained between erythrocyte formation and the hemolysis of cells. If hemolysis is excessive from any cause, an anemia results.

A normal mature erythrocyte is a discoid cell composed of water, protein, lipid, and salts. Ponder,¹ after reviewing various theories concerning its structure, concludes that, "The conception that the red cell possesses a distinct structural membrane of considerable strength composed of protein, lipid, and perhaps other substances, that the interior of the cell contains a fine network, and that the hemoglobin is contained in the meshes of this fine stroma, perhaps partially adsorbed to it, meets all arguments." Ponder² emphasizes that this shape makes it necessary to consider the cell as being in a state of considerable and permanent strain. This strain may be due to the surface membrane of protein and lipoids which cannot be demonstrated histologically, or be dependent on the nature of the internal stroma. When the strain is released, the cell assumes a spherical form, as a result of the action of surface tension.

Every red blood cell wears out in time. The exact span of life is unknown. Perhaps thirty days is an average. The precise factors influencing the normal termination of the activity of an erythrocyte and the method of its disposal are not clear. The spleen plays a major role in destruction and seems to be the graveyard for the worn-out cell. When a cell has been injured by the normal wear and tear of use, or by some toxic agent, the cell tends to become spherical from the loss of the surface or intracellular forces which normally hold it as a biconcave disk. Such a change seems the first stage of hemolysis. There is much evidence that a substance, lysolceithin,³ which is formed in the spleen and makes erythrocytes spherical, aids in the normal hemolysis of old and injured cells and, at times, may be a factor in abnormal hemolysis.

The erythrocytes are taken out of circulation by the reticulo-endothelial cells. The cell stroma is completely broken up. The hemoglobin contained in the stromatin does not return to the blood as such but is disintegrated by the reticulo-endothelial cells. The globin-hematin combination in the hemoglobin molecule is split up, the iron in the hematin fraction is set free, and bilirubin is formed.

*From the Cleveland Clinic.

There are no end products of the stroma which can be identified in the laboratory. The hemoglobin is quantitatively converted into bilirubin, however, so if the amount of bilirubin formed is known, the amount of hemoglobin destroyed and, indirectly, the number of erythrocytes lost, can be calculated. The only exact way to measure bilirubin so formed would be to analyze the total bile excretion. However, there is no practical way to collect this. Fortunately, it is difficult for the liver or the kidneys to excrete the bilirubin formed from hemoglobin because it is bound to protein. With obstructive jaundice there is a retention of bile salts as well as bilirubin. These raise the surface tension and thus free the bilirubin from combination with protein and make it easy to excrete. Since the bilirubin formed from the hemoglobin set free when erythrocytes are hemolyzed tends to be retained, the amount and rate of red blood cell destruction are indirectly determined by measuring the amount of pigment in the plasma, provided there is no disease of the biliary tract.

In almost every hemolytic anemia due to the excessive destruction of erythrocytes, the bone marrow tries to compensate for the increased loss of cells. It is possible that the increase in cell destruction may be fully compensated for by a higher rate of delivery of new cells from the marrow. It is difficult and rare for this to continue. Even with the most active marrow there is usually still an anemia. The usual finding in a hemolytic anemia is both an increase in the bile pigments of the plasma paralleling the rate of cell destruction, and a high reticulocyte count, indicating that cells are being delivered very rapidly from the marrow. The red blood cell count and hemoglobin measure only the balance between formation and destruction at the time the count is made. The reticulocyte count, together with the total red blood cell count, gives indirectly the survival time of the erythrocyte in the circulation. Thus, with a normal red blood cell count of five million per cubic millimeter and a reticulocyte count of 0.5 to 1 per cent, the mean span of life of the red corpuscle is about thirty days. If the reticulocyte count is high, such as 15 to 25 per cent, it is apparent that the mean span of life can be only one to three days.

Since the cells are being removed largely by the spleen, this organ is nearly always enlarged from overactivity. The clinical picture, then, of a hemolytic anemia is anemia with jaundice and an enlarged spleen, with absence of obstruction of the bile ducts. On examination of the blood, besides the anemia, the bile pigments of the plasma are increased, the reticulocyte percentage is increased, and usually there are numerous red blood cells with nuclei, diffuse basophilia, and nuclear remains, such as Isaacs' granules and Howell-Jolly bodies.

Since the bone marrow is overactive in supplying an excessive number of red blood cells, marrow which is normally yellow becomes red through active erythropoiesis. In patients dying of a hemolytic anemia, which has persisted long enough for the low bone marrow to react, the marrow throughout the body may be red. A bone marrow puncture shows excessive erythropoiesis also, as indicated by the large number of cells of the erythrocyte series (Fig. 1).

Abnormal hemolysis may be due to the presence of agents toxic for the erythrocyte in the blood plasma. These may be: (1) toxins resulting from bacterial growth; (2) immune hemolysins, such as occur in the transfusion of

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incompatible blood groups or in paroxysmal hemoglobinuria from cold; or (3) chemical poisons, such as the phenylhydrazine group or snake venoms. In the group of chemical poisons may be included those formed in the body as an allergic response to an antigen, as in favus-bean poisoning. The mechanism of red blood cell destruction by malarial and other intracellular parasites is obvious though different from the preceding group. Damaged cells are taken out of circulation by the spleen. Many other chemical substances will actively destroy red blood cells. Among these are acids, alkalies, bile salts, saponin, and snake venoms. Hemolysis is rapid in hypotonic salt solutions.

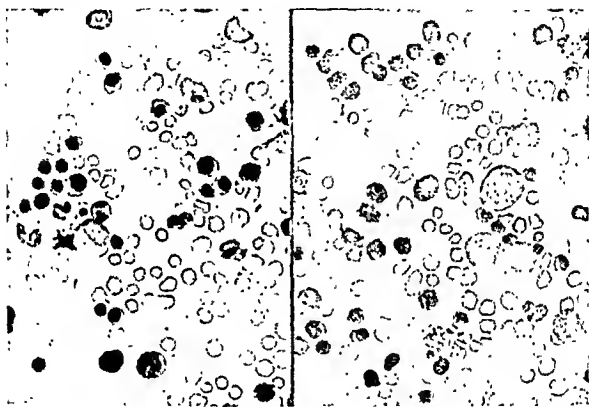


Fig. 1.—Film of marrow obtained by sternal puncture. Note the large number of immature cells of the erythrocyte series.

An increased hemolysis of cells may also result from a defect in the cell itself, rendering it more easily destroyed. The defect may be an abnormal shape of the erythrocyte or an imperfection in quality of the cell stroma which makes it more easily destroyed. Sometimes abnormal hemolysis seems to be due to a perverted activity of the reticulo-endothelial cells, especially in the spleen.

It is difficult to make a satisfactory clinical classification of the hemolytic anemias. One possible grouping is as follows:

- I. Increased hemolysis from excessive destruction of cells damaged by:
 - A. Chemical agents, as phenylhydrazine.
 - B. Bacterial toxins, as in gas gangrene.
 - C. Immune hemolysins, as in transfusion reactions.
 - D. Parasites, as in malaria.
- II. Increased hemolysis due to excessive removal of congenitally abnormal cells:
 - A. Congenital abnormality of shape.
 1. Spherocytic anemia (congenital hemolytic jaundice).
 2. Sick-cell anemia.
 3. Oval-cell anemia.

- B. Congenital abnormality of structure.
 - 1. Erythroblastic anemia of Cooley.
 - 2. Sickle-cell anemia.

- III. Increased hemolysis from abnormal hemolytic activity of spleen or other parts of reticulo-endothelial system.
- IV. Increased hemolysis from unknown cause, as in paroxysmal nocturnal hemoglobinuria (Marchiafava-Micheli type) and the idiopathic types of hemolytic anemia.

CHANGES IN MEASUREMENT OF THE ERYTHROCYTE OF MAN ON HEMOLYSING IN HYPOTONIC SODIUM CHLORIDE SOLUTION

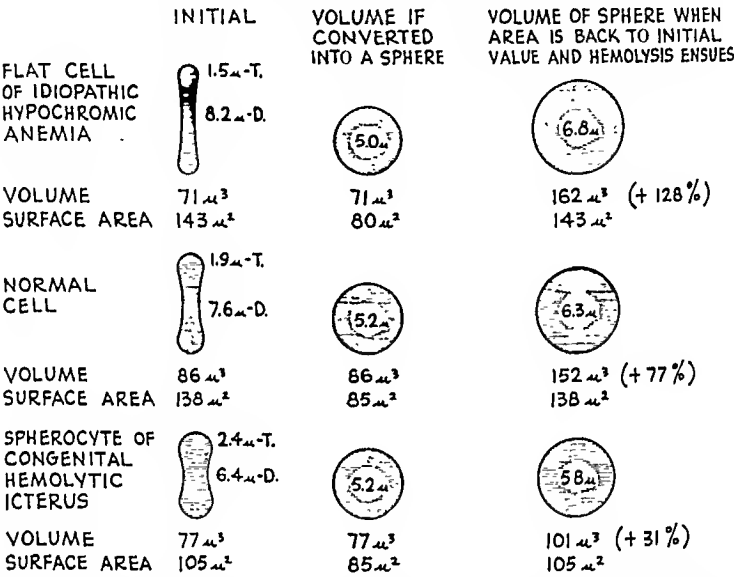


Fig. 2.—Changes in measurement of the erythrocyte of man on hemolyzing in hypotonic sodium chloride solutions. The flatter the cell, the greater the volume the cell can reach before the original surface area is reached as the cell becomes spherical and increases in volume.

The exact mechanism of hemolysis varies with the hemolyzing agent. In hypotonic salt solutions the contents of the cell are altered by the excess of water. The cell becomes spherical, the membrane becomes permeable, and the cell swells as water is taken up. In time, the cell ruptures. Such is the mechanism of hypotonic hemolysis. With hemolysis by a chemical agent, some constituent of the cell is affected so that the structure is altered and the cell breaks up. Thus, saponin and bile salts act on the protein of the cell, while snake venom affects the lecithin and produces lysolecithin, an active hemolytic agent. In every instance there is an alteration of cell structure. If the cell is abnormal at the start, it is apparent that the hemolysis may take place much more easily than in a normal erythrocyte.

With hypotonic hemolysis, the cell changes from a biconcave disk to a sphere during the process of dissolution.⁴ The flatter the cell, the greater the increase in volume possible before the cell membrane, which cannot be stretched, will rupture. Thus a flat cell, with a volume of 71 cubic microns, a diameter of 8.2 microns, and a thickness of 1.5 microns, can increase in volume 128 per cent

before the membrane ruptures. A normal cell will increase 77 per cent. On the other hand, a cell with a volume of 77 cubic microns, a diameter of 6.4 microns, and a thickness of 2.4 microns, can be increased in volume only 31 per cent before the cell is a sphere and further tension ruptures the membrane (Fig. 2). It is this fact that explains the increased fragility in hypotonic solutions of the microspherocytes characteristic of congenital hemolytic jaundice.⁵ Such cells cannot have the normal increase in volume without putting the membrane on tension sufficient to rupture it.

Whenever the cell structure is altered in other ways, there is a tendency for the cell shape to change from the biconcave form to the sphere. The action of lysolecithin, which is normally present and which may be the normal agent in red blood cell hemolysis, is to make the cells spherical.³ Likewise, spherocytosis may be characteristic of the hemolytic anemia due to leucemia, Hodgkin's disease, or acetylphenylhydrazine. On the other hand, the most marked hemolytic anemia may occur without spherocytosis (Table IV). Here the cells break up without going through changes in shape already described. Perhaps here the essential element is overactivity of reticulo-endothelial cells which destroy erythrocytes without changing them chemically. Ham⁶ has shown recently that a change in reaction may also greatly influence the speed with which cells break up.

The two factors in increased hemolysis may be summarized:

- I. Increased hemolysis of normal cells damaged by foreign agents, namely:
 - a. Chemical poisons, such as phenylhydrazine, sulfanilamide, or snake venom.
 - b. Parasites, as the malarial protozoan.
 - c. Bacterial toxins, as in gas gangrene.
 - d. Amboceptor and complement reactions, as in transfusion of incompatible blood, or in paroxysmal hemoglobinuria.
- II. Increased activity of the spleen.

The action of the spleen may be the normal phagocytic activity on damaged cells as described above, or on abnormally shaped, or imperfect cells as in spherocytosis (congenital hemolytic icterus), sicklecytosis, or ovalocytosis. In some cases there seems a true perverted activity of the spleen and perhaps of other parts of the reticulo-endothelial system, as in Hodgkin's disease and leucemia. Thus, the shape and structure of the cell, the activity of the spleen, and the presence of substances toxic for erythrocytes must be considered as possible factors in the production of every hemolytic anemia.

Congenital hemolytic icterus is the best known example of a chronic hemolytic anemia. This disease may continue active for many years. Here there is characteristically a chronic anemia, with acute exacerbations, jaundice, reticulocytosis, and an enlarged spleen. All the signs and symptoms of the disease are due to excessive destruction of red blood cells. The erythrocytes have two distinguishing characteristics. In the first place, they are thicker than normal in relation to diameter. Since the diameter is usually less than normal with little variation from normal in volume, the cells are usually spoken of as micro-

spherocytes. Some of the cells are true spherocytes, but most of them are simply thicker than normal (Fig. 3). All observers are agreed on this fundamental characteristic of the cell in congenital hemolytic icterus. The second characteristic property of the red blood cell is its increased fragility in hypotonic solutions of sodium chloride. This finding is less characteristic than the spherocytosis, since factors other than shape of the cell may influence fragility. A thick cell with a more than resistant envelope may show little if any change in fragility. The cell measurements in ten typical cases of congenital hemolytic icterus are shown in Table I.

TABLE I
THE ERYTHROCYTE IN CONGENITAL HEMOLYTIC ICTERUS

NO.	COUNT	MEAN VOLUME	MEAN DIAMETER	MEAN THICKNESS	VOLUME THICKNESS INDEX	DIAMETER-THICKNESS RATIO	RETICULOCYTES	FRAGILITY	ICTERUS INDEX
	millions	cubic microns	microns	microns			per cent	% $\frac{\text{NaCl}}{100}$	units
1.	2.65	80	6.4	2.5	1.51	2.6:1	10.5	58-39	35
2.	1.82	77	6.5	2.3	1.44	2.8:1	22.1	68-38	20
3.	3.73	84	6.0	3.0	1.73	2.0:1	6.5	68-38	15
4.	3.08	87	6.6	2.5	1.54	2.6:1	14.9	66-40	15
5.	2.13	76	6.4	2.4	1.46	2.7:1	14.7	64-36	25
6.	2.37	111	7.3	2.6	1.45	2.8:1	31.0	56-42	15
7.	2.65	105	7.5	2.4	1.27	3.1:1	10.8	56-38	5
8.	3.07	91	6.5	2.7	1.67	2.4:1	17.9	45-34	25
9.	4.43	85	7.2	2.1	1.18	3.4:1	3.7	44-32	8
10.	3.99	85	6.8	2.4	1.38	2.8:1	4.8	47-39	6

As the name indicates, there is a large hereditary element in spherocytosis. Cases may be sporadic, however, without any evidence of the disease in relatives. The degree of spherocytosis may vary greatly. Thus, in a recently case, a 2-year-old child had an extreme spherocytosis, while the mother had only a mild spherocytosis which was revealed by careful cell measurements. The congenital defect is really an anatomic one, since it represents a variation in the normal shape. It is often accompanied by other anatomic defects, especially steple skull.

From the clinical standpoint, the important features of congenital hemolytic icterus or spherocytic anemia are the chronic anemia, the constant, though mild, jaundice, and the enlarged spleen. Infections and other unknown factors may greatly speed up the hemolytic process, causing the so-called crises during which the anemia is more severe, the icterus is more intense, and the spleen is larger. If there is a family history of jaundice and an enlarged spleen, the picture is complete.

The two characteristic findings in relation to the red blood cell already mentioned, namely, the spherocytosis and the increased hypotonic fragility, have usually been considered as separate defects of the red blood cells. Chauffard⁷ regards these two features as evidence of the lessened vitality of the cells. Meulengracht⁸ is of the opinion that they are regeneration phenomena secondary to the increased bone marrow activity. Gänsslen⁹ expressed the belief that there is a primary defect in the marrow which makes it incapable of supplying cells of normal size and resistance. Naegeli¹⁰ always contended that the defects repre-

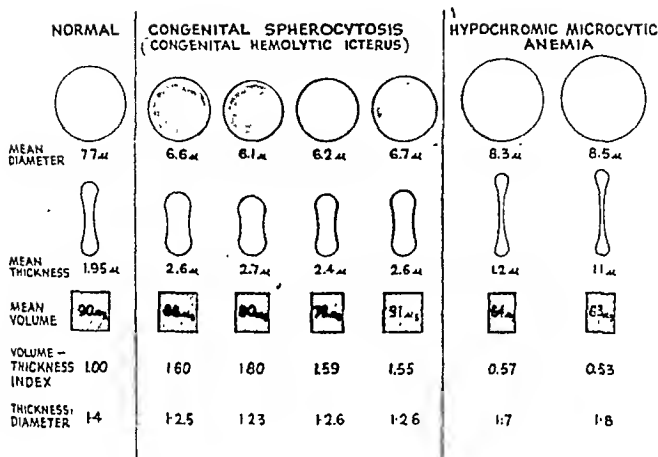


Fig. 3.—The mean red blood cell in congenital hemolytic icterus compared with the normal biconcave disk and the flat, "wedding-ring" erythrocyte of hypochromic microcytic anemia. Note the characteristic increases in thickness, decrease in diameter, with little change in volume.

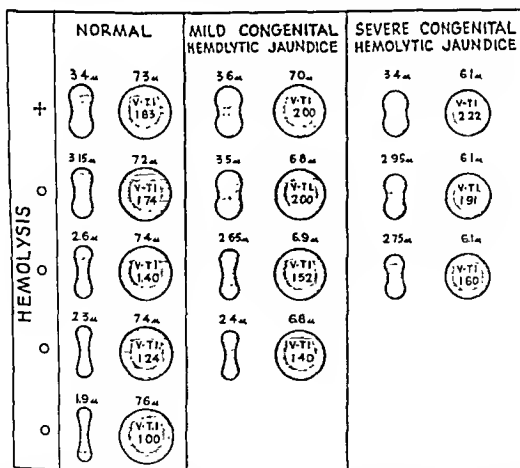


Fig. 4.—Changes in the shape of the erythrocyte in congenital hemolytic icterus compared with the normal in hypotonic hemolysis. The more marked the spherocytosis, the closer the cell is to the hemolysis point. The fragility thus varies directly with the degree of spherocytosis.

sent a congenital anatomic abnormality of the erythrocyte comparable with other anatomic congenital defects. No one seems to have correlated the spherocytosis and the increased fragility.

There is a direct relationship between the shape of the cell and hemolysis.⁵ Ponder has demonstrated that this depends fundamentally upon how much the volume of the cell can increase without stretching and rupturing the covering membrane.¹¹ This point is best illustrated by comparing the changes in a normal red blood cell and that of congenital hemolytic icterus (Fig. 2) in hypotonic solutions of sodium chloride. Note again the increase in volume by immersing in hypotonic solution of 77 per cent (from 86 μ^3 to 152 μ^3) of the normal cells as compared with 31 per cent in a patient with congenital hemolytic icterus. The intensity of congenital hemolytic icterus varies directly with the degree of spherocytosis. The more spherocytic the cell, the nearer it is to the hemolysis point (Fig. 4).

The relation of the shape of the cell to hypotonic fragility is well illustrated by the study of the erythrocytes of different animals. It has long been known that the fragility of the red blood cells in hypotonic solution varies greatly in different animals. Vallery-Radot and Lheritier¹² first pointed this out and concluded that the fragility parallels the diameter of the cells. In Table II are shown the measurements of the mean cell of a group of animals as measured by Emmons,¹³ and the fragility for the same animals as determined by Vallery-Radot and Lheritier. It is apparent that the fragility varies with the globularity of the mean cell; hence there must be a direct relationship of the two factors.

TABLE II
RELATION OF SHAPE OF ERYTHROCYTE TO HYPOTONIC HEMOLYSIS

SPECIMEN	MEAN DIAMETER	MEAN THICKNESS	MEAN VOLUME	VOLUME- THICKNESS RATIO	INITIAL AND MAXIMAL RESISTANCE
	microns	microns	cubic microns		% NaCl
Man	7.8	1.84	88	4.2:1	0.42-0.48
Dog	7.2	1.70	69	4.2:1	0.50-0.54
Rabbit	6.6	1.84	63	3.6:1	0.52-0.54
Cat	5.6	1.75	43	3.2:1	0.60-0.66
Goat	4.0	1.95	25	2.1:1	0.72-0.74

Spherocytosis is the one absolutely constant finding in our group of 30 cases of congenital hemolytic icterus. The diagnosis is not justified unless this characteristic cell is found. The seriousness of the disease can be measured by the extent of the spherocytosis. Is this shape related to increased removal of cells from the blood stream by the spleen in congenital hemolytic icterus? The immediate cause of the anemia is hyperactivity of the spleen, since the symptoms of the disease are all relieved by splenectomy. Spherocytosis always persists after removal of the spleen, although it is less marked than before operation.

The findings in eight patients studied after splenectomy are shown in Table III. In every instance the anemia, jaundice, and reticulocytosis were entirely relieved. The decreased cell diameter and the spherocytosis were still present, though to a less marked degree than before operation. It is apparent

TABLE III
CHANGES IN ERYTHROCYTE AFTER SPLENECTOMY FOR CONGENITAL, HEMOLYTIC ICTERUS

NO.	TIME AFTER OPERATION	COUNT	MEAN VOLUME	MEAN DIAMETER	MEAN THICKNESS	VOLUME-THICKNESS INDEX	DIAMETER-THICKNESS RATIO	RETICULO-CYTES	FRAGILITY	ICTERICUS INDEX
		millions	cubic microns	microns	microns			per cent	% $\frac{\text{NaCl}}{100}$	units
1.	Before	3.20	77	6.4	2.4	1.57	2.7:1	9.4	50-40	15
	7 years	4.99	85	6.9	2.3	1.31	3.0:1	0.5	56-40	6
2.	Before	4.73	79	6.4	2.5	1.53	2.6:1	1.3	61-32	25
	4 years	5.58	86	6.7	2.5	1.13	2.7:1	0.5	51-36	8
3.	Before	2.65	80	6.4	2.5	1.51	2.6:1	10.5	58-39	25
	2 years	4.82	84	6.7	2.4	1.40	2.8:1	0.5	51-34	4
4.	Before	3.07	91	6.5	2.7	1.67	2.7:1	18.0	45-34	25
	2 years	3.04	88	7.1	2.3	1.33	3.1:1	0.5	50-38	1
5.	Before	1.82	77	6.5	2.3	1.44	2.8:1	22.0	69-38	20
	2 years	4.98	80	6.5	2.4	1.48	2.7:1	0.5	52-38	1
6.	Before	4.03	87	6.7	2.5	1.49	2.7:1	6.3	50-42	15
	1 year	5.33	82	6.8	2.3	1.52	3.0:1	0.5	56-43	10
7.	Before	3.73	84	6.0	2.0	1.73	2.0:1	6.5	68-38	15
	8 months	5.33	78	6.2	2.6	1.61	2.4:1	0.5	55-39	5
8.	Before	2.76	92	6.4	2.9	1.77	2.2:1	16.0	68-40	15
	3 months	4.59	82	7.2	2.0	1.12	3.6:1	0.5	56-40	4

from the beneficial results of splenectomy that the anemia is due to the activity of the spleen in filtering out spherocytes. The exact mode of action of the spleen is unknown. The spleen might make the cells more spherocytic and thus more susceptible to hemolysis, perhaps through the action of lysolecithin, as suggested by Bergenhem and Fahraeus.³ Recently I measured the diameter of the cells on a film of blood obtained from the pulp of a spleen immediately after removal for congenital jaundice. The mean cell diameter was 5.8 microns, while the mean diameter of the circulating cells determined at the same time was 6.1 microns. Gripwall¹⁵ has shown also that the cells from the splenic vein show a more pronounced spherocytosis and increased fragility as compared with peripheral blood.

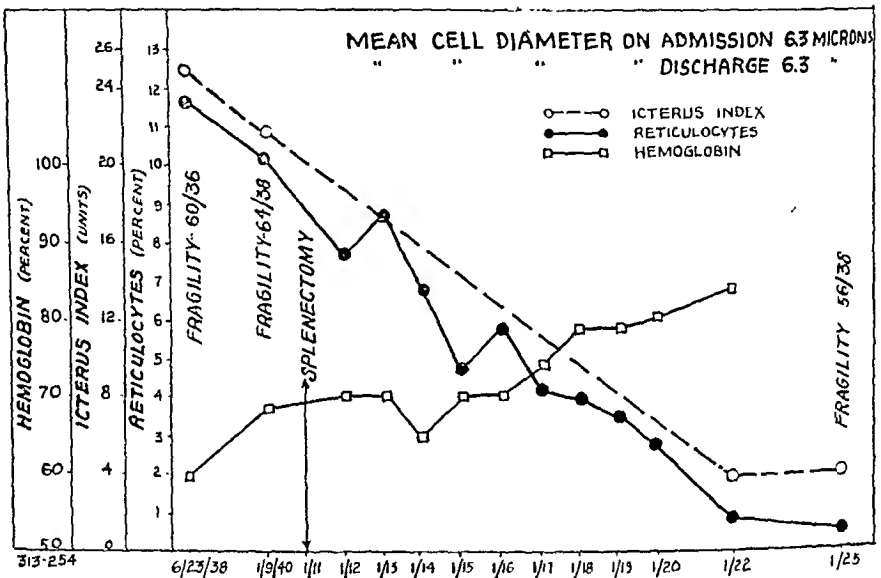


Fig. 5.—The characteristic changes in the blood after splenectomy for congenital hemolytic icterus. Note the fall in the icterus index to normal and the disappearance of the reticulocytosis. The hypotonic fragility changes very little. The hemoglobin gradually rises.

The characteristic changes in the blood after splenectomy for congenital hemolytic icterus are shown in Fig. 5. The icterus index returns to normal, and the reticulocytosis disappears quickly. There is a gradual steady rise in hemoglobin. The increased fragility of the red blood cells persists to a lesser degree. Heilmeyer¹⁷ has made the same observations. The spleen might also affect the cells in some other way so as to injure the cell envelope and thus make it more susceptible to hemolysis. The spherocytes of congenital hemolytic icterus are more susceptible to destruction by heat than are normal cells. The blood of three patients who had had a splenectomy for congenital hemolytic icterus, the blood of two patients who were suffering from the disease but who were not splenectomized, and the blood of a normal individual were heated in a water bath for three hours. There was only a trace of hemolysis in the normal blood. The blood of the splenectomized patients showed well-marked hemolysis, but much less hemolysis than the blood of the patients with the active disease. The mean cell diameter in the splenectomized patients was 6.9, 6.7, and 6.8, with

a volume thickness index of 1.31, 1.43, and 1.33. The mean cell diameter of the other patients was 6.6 and 6.4, with a volume thickness index of 1.54 and 1.46. The hemolysis was most marked with the mean cell diameter of 6.4. Another patient with a mean cell diameter of 6.2 showed the most marked hemolysis when his blood was heated for three hours at $-12^{\circ}\text{C}.$, with the control showing only faint hemolysis.

The persistence of the spherocytosis and increased fragility after splenectomy, without clinical evidence of disease, shows that the bone marrow is still supplying cells thicker than normal. The fact that the cells are more spherocytic, more fragile, and more susceptible to hemolysis by heat before the spleen is removed proves that the spleen in some way affects the cell. This may be caused by changing the cell shape only or by affecting the constituents of the cell so that it becomes more spherocytic and less resistant. Perhaps the action of the spleen on the spherocytic cell is the normal action on every cell. In any event, this is sufficient in congenital hemolytic icterus to cause destruction of the cell and a consequent anemia. Hawksley¹⁴ and Gripwall¹⁵ have emphasized that there is a splenic factor, as well as a congenital abnormality of shape, in the causation of the anemia. Doan, Wiseman, and Erf¹⁶ are of the opinion that the spleen is the chief organ in the hemolytic process. Heilmeyer¹⁷ considers the spleen entirely responsible for spherocytosis and increased hemolysis.

There are evidently factors other than shape affecting the fragility of the cell in congenital hemolytic icterus. While there is a striking parallelism between the resistance of the cells to hemolysis in hypotonic sodium chloride solution and the degree of spherocytosis, there are also exceptions to this rule. Thus in one patient with a marked spherocytosis (volume-thickness index 1.67) the red blood cells have repeatedly shown a fragility within the normal limits or little beyond it. It is evident that the envelope of this patient's cells is more resistant than the usual cell with such a shape. The cells of another patient studied immediately after a hemolytic crisis, during which transfusions were given, were more resistant to hypotonic hemolysis than would be expected from the degree of spherocytosis. Later there was a great increase in fragility with little change in cell shape.

While spherocytosis is a constant finding in congenital hemolytic icterus, spherocytosis may be present without increase in fragility, and the increase in fragility may not parallel the spherocytosis. Just what are the factors influencing the strength of the cell membrane or the stability of the stroma making it more resistant to release of strain is not clear. There may well be some qualitative variation in the protein or lipoids of the cell, or even a difference in the thickness of the cell envelope. The normal protective action of the plasma may vary also. Any such changes might afford a protective action against lysis.

In congenital hemolytic icterus there is a microspherocytosis throughout life. After splenectomy the anemia disappears, and the spherocytosis is less marked. Spherocytosis may be seen under other conditions also. I have observed marked spherocytosis in three patients with outspoken leucemia. The fragility of the cells in hypotonic solutions was increased as one would expect from the shape of the cells. In each instance the spleen was enlarged, and in two instances it was removed without influencing the extent of the anemia or the

TABLE IV
HEMOLYTIC ANEMIA NOT DUE TO CONGENITAL HEMOLYTIC ICTERUS

NO.	SEX	RED BLOOD CELL	HEMATO-CRIT READING	VOLUME	DIAMETER	THICKNESS	RED BLOOD CELL THICKNESS	VOL-UME-INDEX	CELL DIAMETER-THICKNESS RATIO	RETICULO-CYTES	FRAGILITY	ICTERUS INDEX	DIAGNOSIS
		million		cubic microns	microns	microns				per cent	% $\frac{\text{NaCl}}{100}$	units	
1. 323-714	F	1.21	14	116	8.6	2.0	0.94		4.3:1	29.0	46-36	45	Chronic gall bladder disease with perforation
2. 293-371	F	1.85	20	107	9.0	1.7	0.71		5.3:1	15.0	48-32	20	Undetermined
3. 208-866	F	1.95	23	118	7.7	2.5	1.31		3.1:1	46.0	48-30	20	Sarcoid (?) of lung
4. 263-407	M	3.07	25	85	7.7	1.8	0.92		4.2:1	5.1	46-32	15	Hodgkin's disease
5. 326-542	M	2.70	24	88	8.0	1.9	0.82		4.2:1	1.1	40-30	15	Hodgkin's disease
6. 310-399	F	2.06	16	79	7.8	1.7	0.84		4.6:1	1.5	42-34	15	Leucemia
7. 279-924	M	1.40	17	122	8.2	2.3	1.12		3.6:1	3.0	42-36	10	Leucemia
8. 296-796	M	3.20	30	95	7.6	2.1	1.10		3.6:1	11.4	41-34	8	Leucemia
9. 317-198	M	1.62	19	118	7.7	2.3	1.31		3.4:1	52.0	72-42	25	Leucemia
10. 234-059	M	2.27	26	100	7.0	2.4	1.48		2.9:1	11.0	64-48		Leucemia
11. 310-809	M	1.43	18	124	7.2	2.7	1.63		2.7:1	48.0	64-40	20	Undetermined
12. 309-885	F	2.10	25	121	8.0	2.4	1.19		3.3:1	17.0	Free hemoglobin in plasma	90	Paroxysmal nocturnal hemoglobinemia
13. 329-115	M	3.59	34	95	8.6	1.7	0.77		5.0:1	4.5	44-32		Paroxysmal nocturnal hemoglobinemia
14. 330-133	F	3.60	36	100	8.1	1.9	1.00		4.1:1	14.1	44-28	45	Idiopathic hemolytic anemia

course of the disease. Another patient with a probable sarcoid of the lung had spherocytosis and increased fragility of varying intensity, depending on the degree of activity of the lung disease. The patient has made a complete recovery. The marked hemolytic anemia has disappeared, as has the spherocytosis. It seems apparent, as emphasized by Ponder, that a toxic process may so affect the cell as to make it spheroid. In the hemolytic anemia produced by phenylhydrazine, I have demonstrated a spherocytosis which is only an evidence of damage to the cell, with release of the internal strain and the taking on of a spherical form.

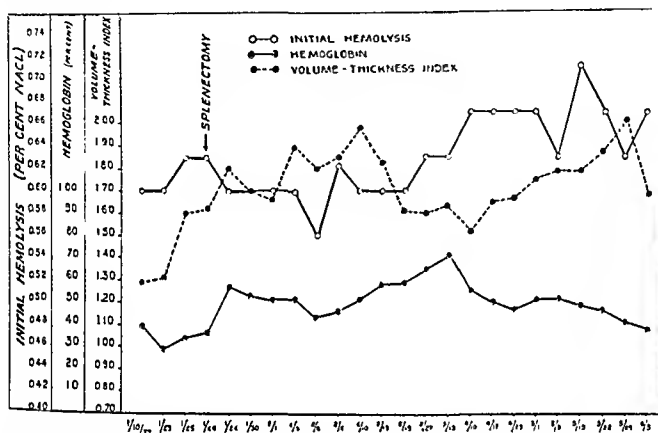


Fig. 6.—Changes in the blood of patient showing acquired spherocytosis of unknown origin after splenectomy. The hypotonic fragility is unchanged. The hemoglobin changes little and the reticulocytosis persists. The splenectomy did not alter the fatal course of the disease. The spherocytosis, as indicated by the volume-thickness index, becomes more marked.

Dameshek and Schwartz¹⁸ studied the changes taking place in the red blood cell when the guinea pig is injected with antiguinea pig hemolytic serum. The cells of the animal injected become spherocytic and show increased fragility in hypotonic sodium chloride solution. They further demonstrated isohemolysins of the immune body type in three patients with acute hemolytic anemia. In one spherocytosis and increase in fragility were marked. All three were relieved by splenectomy. Dameshek and Schwartz conclude from their experimental studies that hemolytic anemias are due to hemolysins and that the spherocytosis is due to hemolysis and not primarily to an abnormal type of cell. These observations only show that spherocytosis can be acquired. It is very doubtful whether they have any application in congenital hemolytic icterus. The spherocytosis here evidently results from the reaction of the cell to the injury by hemolysis.

I recently studied one patient with marked spherocytosis and a great increase in fragility who constantly had large amounts of hemolysin in the blood plasma. When first seen this patient had a marked anemia, his volume thickness index was 1.29, and hemolysis began in 0.60 per cent sodium chloride solution. There

was no family history suggesting congenital hemolytic icterus, the blood of other members of the family was normal, and the patient had been entirely well until the onset of his present illness. The anemia was so marked and the response to therapy so poor that splenectomy was done. There was some improvement for two months, but the reticulocytosis, the hemolysins, the increased fragility, and the spherocytosis persisted. The anemia became progressively worse. The patient died of the anemia five months after the splenectomy. Autopsy showed no trace of a spleen. It is apparent that the spherocytosis and anemia here were not due to the spleen. This patient evidently had an acquired spherocytosis, and the hemolytic anemia was not dependent on any abnormality or activity of the spleen. The changes in the blood (Fig. 6) are in marked contrast to those observed following splenectomy for congenital spherocytosis (Fig. 5).

Sickle-cell anemia and the erythroblastic anemia of Cooley are also good examples of chronic hemolytic anemia. In these anemias there is evidently an inherited defect in quality of the stroma, so that the cells disintegrate and are removed by the spleen. The curious shapes observed in sickle-cell anemia are simply the expression of this abnormality of the stroma. As the stroma changes, especially with anoxemia, the cell takes on the characteristic sickle form. These diseases must be looked upon as congenital defects in quality of the stroma. There may be other unrecognized congenital defects in the stroma.

I have recently observed a mother and two children, all showing an identical hemolytic anemia. The mother's mother had died with jaundice, anemia, and enlarged spleen at an early age. All three patients showed icterus, reticulocytosis, and free hemoglobin in the blood plasma without hemoglobinuria. Repeated study of the red blood cells showed no increase in hypotonic fragility and no spherocytosis. The cells hemolyzed rapidly, however, under conditions in the laboratory which would not produce hemolysis of normal cells. A splenectomy was done for the mother without influencing the hemolytic process (Fig. 7). It seems apparent that the only logical explanation for such findings is a congenital weakness of the erythrocyte which makes it more susceptible to hemolysis. The presence of free hemoglobin in the plasma should have shown that splenectomy was contraindicated.

The anemia of malaria and other protozoan diseases in which the red blood cells are invaded and destroyed by parasites needs no discussion. Likewise, there is nothing unusual about the anemia dependent on the destruction of cells by various poisons which affect the different constituents of the red blood cell. The anemia of snake venom poisoning is one of the most interesting of this group, because it has been proved that the venom removes the unsaturated fatty acid from the lecithin molecule, thus producing lysolecithin which is a violent hemolytic agent.

As red blood cells are destroyed, hemoglobin must be set free. It is a curious fact that even with most marked hemolytic crises in congenital hemolytic icterus no free hemoglobin is ever demonstrated in the blood plasma. Likewise, in a sickle-cell anemia, Cooley's anemia, and the so-called idiopathic hemolytic anemia, free hemoglobin is not demonstrable. In this group of cases it seems apparent that the cells are destroyed within the spleen or other parts of the reticulo-endothelial system. It is in these cells also that hemoglobin is disintegrated with the production of bilirubin.

In another group of hemolytic anemias, however, hemoglobinuria is a striking clinical feature of the patient's disease. Thus, in transfusions with incompatible blood, by the action of isohemolysins, hemoglobin is set free; if it rises above a certain level in the blood plasma, it is excreted by the kidneys. The paroxysmal hemoglobinuria due to cold is again due to the overflow of free hemoglobin through the kidney as a result of the rapid destruction of cells by an amboceptor-complement reaction. In such instances the hemoglobinuria is not due to the hemolysis of cells alone, as with even more rapid destruction in the crises of congenital hemolytic jaundice no hemoglobin is present in the plasma. The essential difference here seems the fact that the hemolysis is taking place in the circulating blood rather than in the spleen or other tissues where the hemoglobin is disposed of without becoming free in the blood plasma. It is apparent that splenectomy is not indicated if the cells are hemolyzing in the plasma unless the spleen is setting free a toxin which has never been proved. It is possible also that the hemolysis in such cases is due to the loss of normal protective power of the blood plasma.

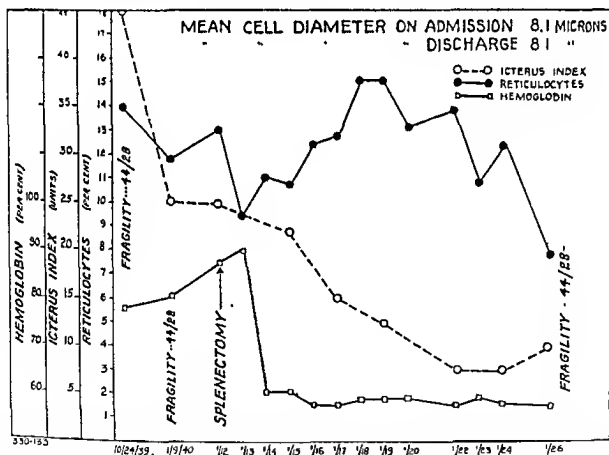


Fig. 7.—Changes in the blood of a patient with hemolytic anemia without spherocytosis. The patient had hemoglobinemia. The splenectomy did not alter the course of the disease. Note the persistence of bilirubinemia and reticulocytosis and absence of rise in hemoglobin.

One type of hemolytic anemia, first described by Marchiafava and Micheli in Italy, seems a definite clinical entity.⁶ In this type the patients have not only hemolytic anemia, but also constant hemoglobinemia and hemoglobinuria. The hemoglobinuria is described as paroxysmal and nocturnal and seems to be characteristic of earlier stages of the disease and of milder cases. I have examined the blood of two patients and observed another. The blood of one patient has been studied repeatedly from every standpoint. Such cases have no family history of the disease. The condition is usually not manifested until after adolescence and may develop at any age. No spherocytosis is present. Ham⁶

has demonstrated that the cells are more susceptible to hemolysis with weak solutions of acids than are normal cells. It seems evident that the disease is inherent in the red blood cells. The defect is certainly in quality and not in shape. The spleen has been removed from several patients without influencing the course of the disease. Here again it seems that the presence of free hemoglobin indicates that the defect is in the red blood cells and is such as to allow them to hemolyze in the circulation. If this is true, a splenectomy should not influence the disease. This has been the experience of everyone with splenectomy in this type of hemolytic anemia. No other treatment has been found of value.

There is a final group of hemolytic anemias which we speak of as idiopathic. Some are probably due to infections; some to the presence of unidentified hemolytic agents. The spleen seems at fault in others. Fortunately, this group is small. Splenectomy is usually justified if hemoglobinemia is not demonstrated. The results are variable.

SUMMARY AND CONCLUSIONS

A hemolytic anemia is due to an excessive destruction of red blood cells beyond the compensatory capacity of the bone marrow to deliver new cells.

The normal red blood cell has a delicate structure which may be injured in many ways. The biconcave shape results in a state of strain in the stroma.

As erythrocytes are destroyed, iron is set free, and bilirubin is formed as the end product of hemoglobin destruction.

The clinical picture of a hemolytic anemia is an anemia with jaundice and an enlarged spleen.

The laboratory study in a hemolytic anemia shows a high reticuloocyte count from excessive marrow activity, a high icterus index, and a hyperplastic bone marrow.

The excessive destruction of red blood cells in a hemolytic anemia may result from (1) damage to the red blood cells, (2) an abnormal shape of the red blood cells, (3) a congenital defect in quality of stroma, or (4) an overactivity of the spleen.

The mechanism of hemolysis varies with the hemolytic agent.

When a cell is injured, it tends to assume a spherical shape since the strain incident to the biconcave shape is released.

The most characteristic and clinically important hemolytic anemia is congenital spherocytosis or congenital hemolytic icterus. The inherited defect here is the abnormal shape of the red blood cell.

A spherocyte is more easily hemolyzed than is a biconcave disk.

There is a direct relationship between hypotonic fragility of the red blood cell in spherocytosis and its shape. A sphere has a smaller surface area in relation to volume, so it can undergo less stretching before hemolysis occurs than a biconcave disk.

The anemia disappears after splenectomy in congenital hemolytic icterus. The spherocytosis persists, but it is less marked.

Spherocytosis may be acquired since an injury to cells by any agent may cause it. Acquired spherocytosis is characterized by increased fragility of cells.

In many hemolytic anemias there is no abnormality of shape so the hemolysis must depend upon other factors, such as an abnormal reaction, changes in the cell membrane, stroma, or constituents of the plasma, or abnormal reticulo-endothelial activity.

In sickle-cell anemia and in the erythroblastic anemia of Cooley, there seems to be a congenital defect in the stroma which allows the cells to be easily hemolyzed abnormally.

If hemoglobinuria is present in a hemolytic anemia, the cells are being destroyed in the circulating blood and not in the spleen.

With hemoglobinemia and hemoglobinuria, splenectomy is contraindicated.

REFERENCES

1. Ponder, E.: Kinetics of Hemolysis, *Physiol. Rev.* 16: 19, 1936.
2. Ponder, E.: The Physical Structure of the Red Cell Membrane With Special Reference to Its Shape, *J. Faraday Society* 33: 947, 1937.
3. Bergenhem, B., and Fuhrerus, R.: Über spontane Hemolysinebildung im Blut, unter besonderer Berücksichtigung der Physiologie der Milz, *Ztschr. f. d. ges. exper. Med.* 97: 555, 1936.
4. Haden, R. L.: The Nature of Hemolytic Anemia. Symposium on the Blood, Univ. Wisconsin Press, pp. 83-104, 1939.
5. Haden, R. L.: Mechanism of Increased Fragility of Erythrocytes in Congenital Hemolytic Jaundice, *Am. J. M. Sc.* 188: 411, 1934.
6. Ham, T. H.: Chronic Hemolytic Anemia With Paroxysmal Nocturnal Hemoglobinuria; Study of Mechanism of Hemolysis in Relation to Acid-Base Equilibrium, *New England J. Med.* 217: 915, 1937.
7. Chauffard, A.: Quoted by E. Meulengracht.⁸
8. Meulengracht, E.: Der chronische hereditäre hämolytische Ikterus. (Konstitutionelle Hypersplenie.) Eine nosographische Studie, Berlin, 1922, Klinkhardt.
9. Günsslen, M.: Der hämolytischen Ikterus, *Arch. f. klin. Med.* 140: 210, 1924.
10. Naegeli, O.: Blutkrankheiten und Blutdiagnostik; Lehrbuch der klinischen Hämatologie, ed. 5, Berlin, 1931, J. Springer.
11. Ponder, E.: Spherical Form of Mammalian Erythrocyte; Changes in Surface Area in Disks and Spheres, *J. Exper. Biol.* 14: 267, 1937.
12. Vallery-Randot, P., and Lherillier, A.: Parallelism Between Globular Resistance to Sodium Chloride Solution and Dimensions of Blood Corpuscles in Mammals, *Compt. rend. Soc. de biol.* 82: 195, 1919.
13. Emmons, W. F.: Interrelation of Number, Volume, Diameter, and Area of Mammalian Erythrocytes, *J. Physiol.* 64: 215, 1927.
14. Hawksley, J. C.: Spherocytosis. Diameter of Erythrocytes in Familial Acholuric, 1936.
15. Gripwall, E.: Hereditären hämolytischen Ikterus, Uppsala, 1938.
16. Doan, C. A., Wiseman, B. K., and Erf, L. A.: Studies in Hemolytic Jaundice, *Ohio State M. J.* 30: 493, 1934.
17. Heilmeyer, L.: Die Sphärocytose als Ausdruck einer Pathologischen Funktion der Milz, *Deutsches Arch. f. klin. Med.* 179: 292, 1936.
18. Dameshek, W., and Schwartz, S. O.: Hemolysis as Cause of Clinical and Experimental Hemolytic Anemias, With Particular Reference to Nature of Spherocytosis and Increased Fragility, *Am. J. M. Sc.* 196: 769, 1935.

PRESERVED BLOOD "BANKS" IN RELATION TO TRANSFUSION IN THE TREATMENT OF DISEASE*

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SINCE blood transfusion has come into wider and wider use not only in the treatment of hemorrhage, surgical shock, and the anemias, but also in the treatment of the prothrombin deficiencies, hemorrhagic dyscrasias, acute and chronic infections, with special reference to the septicemias, as well as many other disease states, and, since the use of preserved blood in "banks" has become increasingly popular because of its several advantages, it is my purpose to discuss briefly and concisely the value of different preservatives and to point out the limitations of preserved blood as a therapeutic agent.

In the United States human blood is commonly preserved in "banks" by the addition of 14 c.c. of a sterile 2.5 per cent solution of chemically pure sodium citrate in physiologic saline solution to each 100 c.c. of blood, with preservation at 4° to 6° C. in a refrigerator. This gives a 0.35 per cent concentration of sodium citrate for the prevention of coagulation, and after typing, sterility, and Wassermann tests, the blood is ordinarily available for transfusion over a period of ten days to several weeks.

Physiologically, and according to clinical experience, such preserved citrated blood is apparently satisfactory for the restoration of blood volume and is superior to saline, glucose, and acaemia solutions in the treatment of severe traumatic hemorrhage and shock. Whether or not the erythrocytes are sufficiently preserved to carry on internal respiration is debatable; likewise the question as to whether or not the platelets and prothrombin are sufficiently preserved for the treatment of the thrombocytopenic purpuras, other hemorrhagic dyscrasias, and the hemorrhage of jaundice.

It is sometimes stated that the chief value of blood transfusion in the treatment of septic states, with or without associated septicemia, is to combat the secondary anemia, and that it is not indicated or required until or unless the hemoglobin is reduced to about 40 per cent, with a corresponding reduction in erythrocytes.

With this I do not agree. Septicemia is apparently the result of a breakdown or exhaustion of the normal clearing mechanism of the blood; not infrequently the result of continuous accession of bacteria and their toxic products from foci of infection of the fixed tissues. I have gradually gained the conviction that one of the most important reasons for frequent transfusions in the treatment of septic states, and especially those with an associated septicemia, is to furnish specific and nonspecific immune substances in the blood of the donor, which may have become reduced or exhausted in the hard-pushed patient or recipient. Furthermore, it is not unlikely that the leucocytes of *fresh* blood

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may serve a useful therapeutic purpose. Therefore, without questioning the value of transfusion in septic states from the standpoint of combating secondary anemia, it seems to me that proper emphasis must be placed upon its possible additional value from the immunologic standpoint, and if this is true, the therapeutic status of preserved citrated blood should be examined from both angles.

Briefly and in general terms, it may be stated that normal or natural resistance and immunity to infection are due in part to specific antitoxins and such sensitizing antibodies as agglutinins, opsonins, and bacteriolysins that may be present in the blood. To these, from the nonspecific standpoint, must be added complement, leukins (from leucocytes), and plukins (from platelets). Among these, the opsonins are of particular importance because of the role they play in phagocytosis, not only by the microphages of the blood and fixed tissues, but also by the cells of the reticulo-endothelial system. Furthermore, agglutinins likewise facilitate both phagocytosis and bacteriolysis in vivo and complement is especially important in both particulars, while leukins and plukins are apparently largely responsible for the normal nonspecific bactericidal activity of the blood. Indeed, it would appear that the chief therapeutic value of non-specific immunotransfusions conducted by the intravenous injection of the donor with 100 million killed typhoid bacilli in vaccine a few hours before transfusion is due in part, both to the transfer of more than normal numbers of leucocytes from donor to patient and to additional amounts of these normal or natural non-specific bactericidal substances.

Certainly we have ample data for indicating that these specific and non-specific mechanisms are responsible for the remarkable capacity of the blood for normally clearing itself of organisms and especially because of phagocytosis by the cells of the reticulo-endothelial system. In septic states, especially septicemia, it is a logical deduction that this clearing mechanism is reduced or exhausted. If this is true, one of the purposes of transfusion in treatment should be to supply the hard-pressed patient with as many of these specific and non-specific factors as possible. One cannot now state that natural antitoxins for the several exotoxins of staphylococci and streptococci are reduced or exhausted in acute infections and especially septicemia, but this may be logically inferred. While it is admittedly difficult to measure complement in relation to bacteriolysis, it is comparatively easy to measure it in relation to hemolysis, and I am convinced on this basis that it is frequently reduced below average normal levels in staphylococcus and streptococcus septicemias. Indeed, I believe that one of the purposes of transfusion is to supply the patient with the complement of the donor; and I believe the same applies to opsonins, which likewise have been found to undergo reduction below average normal levels and especially in acute fulminating infections.

If one of the purposes of transfusion in the treatment of septic states is to supply the patient with these specific and nonspecific immunologic principles, in addition to combating secondary anemia, the question at issue is whether or not transfusion supplies them in amounts of therapeutic value, in view of the fact that normal blood admittedly carries but small amounts of these substances. Indeed, the difference of opinion that exists on the clinical value of transfusion in the treatment of acute and chronic infections is largely on this basis. Insofar as specific antibodies are concerned, it would appear that the blood of con-

valescent or actively immunized donors is to be preferred on the basis of supplying larger amounts, and for this reason specific immunotransfusions are always to be preferred when possible.

Furthermore, and especially in relation to preserved citrated blood in "banks" for the treatment of acute and chronic infections, and particularly septicemias, the important question is whether or not immune bodies, as well as functionally active erythrocytes and platelets, are sufficiently preserved to be of value. Most attention has been given the matter of preservation of erythrocytes and the prevention of hemolysis, but as I pointed out in 1939,¹ blood so preserved showed such rapid disintegration of polymorphonuclear neutrophils with reduction in their phagocytic activity, along with rapid disintegration of platelets and gradual loss in bactericidal activity, that preserved citrated blood was considered inadvisable for the treatment of the anemias, hemorrhagic states, and infections, although it was apparently useful in the treatment of acute hemorrhage and surgical shock.

Recently there has been an increasing appreciation of the fact that if dextrose or dextrin is added to preserved citrated blood, sodium penetration and cell swelling are decreased, with inhibition of hemolysis as originally shown by Rous and Turner.² Thus Maisels and Whittaker^{3, 4} reported, not only these effects following the addition of carbohydrates, but also an increase of surface area of the erythrocytes, so that they can tolerate a larger absolute inflow of fluid. Aylward and his colleagues⁵ found that citrate-glucose preservation is superior to citrate alone in delaying hemolysis and retards chemical changes in the cells, leading to an increase of plasma inorganic phosphate with reduced plasma potassium probably due to a lower degree of hemolysis. DeGowin, Harris, and Plass⁶ likewise found that large amounts of isotonic dextrose reduce hemolysis and that erythrocytes better resist the effects of shaking and agitation. Whether or not efforts toward the prevention of hemolysis in preserved blood is a matter of practical importance, although desirable from the standpoint of therapy, cannot be stated; particularly since O'Shaughnessy and his colleagues⁷ have reported that 5 per cent solutions of hemoglobin in Ringer's solution are capable of carrying oxygen and maintaining osmotic pressure although not capable of maintaining life more than thirty-six hours because of rapid removal by the cells of the reticulo-endothelial system.

Belk, Henry, and Rosenstein⁸ have confirmed my observations on the marked and rapid disintegration of granulocytes and platelets in preserved citrated blood, and have showed increased fragility of the erythrocytes, rapid glycolysis, changes in oxygen capacity, and plasma carbon dioxide. They also observed that the transfusion of hemolyzed blood produced jaundice in some cases. Whether or not preservation with dextrin or dextrose with sodium citrate results in better preservation of granulocytes, platelets, and immunologic properties of plasma has required investigation.

Under the circumstances I have thought it advisable to determine the properties of four well-known preservatives (two without and two with carbohydrate) in relation to the fragility, dehemoglobinization, and disintegration of erythrocytes; and in relation to the preservation of leucocytes, with special reference to the polymorphonuclear neutrophils, platelets, prothrombin time, isoagglutinins, complement, and bactericidal activity. Some attention has been

given prothrombin, as based upon examinations employing the method of Howell, but the technique employed for the collection and preservation of blood did not permit the use of the method of Quick. Nor have I included determinations for potassium, since DeGowin and his colleagues⁴ have shown that there is progressive diffusion from human erythrocytes into plasma, reaching the maximum in from fifteen to twenty days, and because variations in sodium, chloride, citrate, and dextrose in preservatives, as well as different temperatures and atmospheres, did not affect the rate of diffusion which cannot be explained by the release of this ion from completely hemolyzed corpuscles. Furthermore, it was found in blood preserved for as long as thirty days that the diffused potassium was neither toxic nor high enough to cause significant changes in the serum potassium of the recipient.¹⁰

Two adult donors were selected belonging to groups A and B and the blood of each was used with the following four preservatives:

(a) *Sodium Citrate (citrate):*

Sodium citrate (dihydric)	2.5 Gm.
Distilled water	100.0 c.c.

14 c.c. of the solution are used with 100 c.c. of blood, giving a final concentration of 0.35 per cent citrate.

(b) *Moscow Institute of Hematology (M.I.H.), as given by Elliott¹¹:*

Sodium chloride	3.5 Gm.
Sodium citrate (dihydric)	2.5 Gm.
Potassium chloride	0.1 Gm.
Magnesium sulfate	0.002 Gm.
Distilled water	500.0 c.c.

100 c.c. of the solution are used with 100 c.c. of blood.

(c) *Modified Ross-Turner (R-T), as described by DeGowin, Harris, and Plasse⁶:*

Solution A: 3.2 Gm. sodium citrate (dihydric) dissolved in 100 c.c. of distilled water.

Solution B: 10.8 Gm. dextrose dissolved in 200 c.c. of distilled water.

For use, 20 c.c. of solution A and 130 c.c. of solution B are mixed with 100 c.c. of blood.

(d) *Maisels and Whittaker (M-W),¹² as described by these authors:*

Sodium chloride	0.43 Gm.
Sodium citrate (dihydric)	1.05 Gm.
Dextrin	8.5 Gm.
Distilled water	100.0 c.c.

For use, 50 c.c. of the solution are mixed with 100 c.c. of blood.

Before use each preservative solution was sterilized in the Arnold sterilizer for one hour on each of two successive days and each was examined for sterility by aerobic and anaerobic cultures.

The mixtures of blood and preservative were kept in a refrigerator at 4° to 6° C., and the following ten examinations were conducted with each within twenty-four hours, three, five, seven, ten, fourteen, and twenty-one days after collection:

1. Macroscopic record of spontaneous dehemoglobinization.
2. Fragility or tonicity of erythrocytes.
3. Total erythrocyte counts.
4. Total leucocyte counts and morphologic changes in the polymorphonuclear neutrophils.
5. Differential leucocyte counts.

6. Platelet counts by the smear method.
7. Prothrombin time of the plasma (Howell method).
8. Preservation of isoagglutinins in the plasma.
9. Complement content of the plasma for sensitized sheep corpuscles.
10. Bactericidal activity of the plasma for *B. typhosus*.

As previously stated, determination of prothrombin time (method of Howell) was used as the method of Quick for prothrombin could not be employed owing to technical conditions. Rhoads and Panzer¹³ stated that blood preserved for a week or more is practically useless in the treatment of acute prothrombin deficiency encountered in jaundiced patients not adequately treated with vitamin K and bile salts. In their opinion, blood in "bank" for three days would probably be of some slight value, but would be so inferior to fresh blood that they recommend that only the latter be used when transfusion is intended to combat the hemorrhagic tendency in jaundice. Ziegler¹⁴ also found that prothrombin decreased over a period of time in preserved citrated blood kept at 34° F. to a level of 40 per cent of original content and states that in cases in which it is desired to raise the prothrombin content of the blood by transfusion, old "banked" blood is not a suitable agent. Quick¹⁵ likewise found that the prothrombin diminishes in decalcified blood and that it is inferior to fresh blood for controlling hemorrhage in jaundice. However, the matter may be of more academic value than practical interest, since it is thought that transfusion even with fresh blood probably raises the prothrombin content of the blood of the recipient by only 6 to 8 per cent.

Not without interest was the question of the persistence of isoagglutinins for erythrocytes in preserved blood in relation to transfusion. Strumia, Wagner, and Monaghan¹⁶ state that the intravenous injection of stored plasma from citrated blood (100 c.c. of 2 per cent citrate to 500 c.c. of blood) at the rate of 5.6 c.c. per minute produces no reactions, even though it is capable of agglutinating the erythrocytes of the recipient in dilutions as high as 1:80. Knott and Koerner,¹⁷ however, state that citrated plasma may retain agglutinins for several weeks when kept at a low temperature. They think it is advisable to test the erythrocytes of the recipient with a 1:50 dilution of stored O plasma before administration, because O plasma sometimes contains agglutinins a and b in high concentration, capable of quickly agglutinating the corpuscles of groups A, B, and AB.

The results of this investigation¹⁸ have shown that:

1. The two carbohydrate preservatives gave better protection of erythrocytes against dehemoglobinization, fragility, and disintegration than the plain citrate and Moscow Institute of Hematology preservatives.
2. Marked reduction of the total leucocytes occurred with all four preservatives within three days, especially due to disintegration of the polymorphonuclear neutrophils, but the two carbohydrate preservatives gave somewhat better preservation than the two without dextrose or dextrin.
3. Marked reduction in the platelets occurred with all four preservatives within three days, but both carbohydrate preservatives gave somewhat better preservation than the two without dextrose or dextrin.

4. A slight decrease in prothrombin time (Howell) was observed with the plasmas of all preserved bloods within the first twenty-four hours of collection of blood, presumably due to disintegration of platelets. During the following three to seven days this was followed by an increase, presumably due to loss of prothrombin.

5. The isagglutinins showed no decrease during the first ten days with all four preservatives, but showed some diminution after fourteen and twenty-one days, which was somewhat less marked with the two carbohydrate preservatives.

6. Complement was well preserved by all four preservatives for the first seven to ten days.

7. The bactericidal activity of the plasmas for *B. typhosus* was well preserved by all four preservatives for seven days, following which rapid deterioration occurred.

It is apparent, therefore, that none of the four preservatives employed in this investigation afford adequate protection of the polymorphonuclear neutrophilic leucocytes and platelets, and that this phase of blood preservation has not received the attention it deserves in relation to the transfusion treatment of the acute and chronic infections as well as, possibly, in relation to the hemorrhagic states due to prothrombin deficiency.

Possibly the same applies to the transfusion treatment of the anemias, although preserved blood appears adequate for the treatment of acute hemorrhage and surgical shock. For these purposes Macdonald and Stephen¹⁹ join me in the belief that the addition of dextrose (modified Rous-Turner) or dextrin (Maisels and Whittaker) to the preservative is advisable as they both appear to preserve erythrocytes better than plain citrate or the preservative advocated by the Moscow Institute of Hematology. Boland, Craig, and Jacobs²⁰ believe that the M.I.H. preservative is advisable for long preservation and the plain citrate for short preservation. With these conclusions I cannot agree. I advise that blood preserved for more than three days, although apparently satisfactory for the treatment of acute hemorrhage and surgical shock, should not be used in the transfusion treatment of acute and chronic infections, and the anemias and hemorrhagic states.*

REFERENCES

1. Kolmer, J. A.: Preserved Citrated Blood "Banks" in the Treatment of Disease With Special Reference to the Immunologic Aspects, *Am. J. M. Sc.* 197: 442, 1939.
2. Rous, P., and Turner, J. R.: The Preservation of Living Red Blood Cells in Vitro. I. Methods of Preservation, *J. Exper. Med.* 23: 219, 1916.
3. Maisels, M., and Whittaker, N.: Effects of Storage on Human Erythrocytes, *Lancet* 1: 113, 1940.
4. Maisels, M., and Whittaker, N.: Diluents for Stored Blood, *Lancet* 1: 590, 1940.
5. Aylward, F. X., Mainwaring, B. R. S., and Wilkinson, J. F.: Effects of Some Preservatives on Stored Blood, *Lancet* 1: 685, 1940.
6. DeGowin, E. L., Harris, J. E., and Plass, E. D.: Studies on Preserved Human Blood. I. Various Factors Influencing Hemolysis, *J. A. M. A.* 114: 850, 1940.

*An additional advantage in the use of preserved blood may be greater safety from the danger of transfusion syphilis. I have inoculated 10 c.c. of fresh citrated blood with 1 c.c. of a heavy suspension of virulent *Treponema pallidum* (Nichols-Hough strain) from acute testicular syphilomas of rabbits showing approximately 200 treponemes per dark field. A rabbit inoculated at once with 1 c.c. of the citrated-blood-treponeme mixture intratesticularly, as well as rabbits inoculated one and three hours later, developed acute testicular syphilis in about five to six weeks. Rabbits inoculated one, two, seven, fourteen, and twenty-eight days later (the mixture being kept at 4° to 6° C.) escaped testicular infection, and lymph gland transfers to fresh rabbits made six weeks later were negative. The results indicate, therefore, that *Treponema pallidum* in citrated blood may die after twenty-four hours of preservation at 4° to 6° C.

7. O'Shaughnessy, L., Mansell, H. E., and Stone, D.: Hemoglobin Solution as a Blood Substitute, *Lancet* 2: 1068, 1939.
8. Belk, W. P., Henry, N. W., and Rosenstein, F.: Observations on Human Blood Stored at 4 to 6 Degrees Centigrade, *Am. J. M. Sc.* 198: 631, 1939.
9. DeGowin, E. L., Harris, J. E., and Plass, E. D.: Studies on Preserved Human Blood. II. Diffusion of Potassium From the Erythrocytes During Storage, *J. A. M. A.* 114: 855, 1940.
10. DeGowin, E. L., Hardin, R. C., and Harris, J. E.: Studies on Preserved Human Blood. III. Toxicity of Blood With High Plasma Potassium Transfused Into Human Beings, *J. A. M. A.* 114: 858, 1940.
11. Elliott, G. A., MacFarlane, R. G., and Vaughan, J. M.: The Use of Stored Blood for Transfusion, *Lancet* 1: 384, 1939.
12. Maisels, M., and Whittaker, N.: Preservation of Stored Blood, *Lancet* 2: 1219, 1939.
13. Rhoads, J. E., and Panzer, L. M.: The Prothrombin Time of "Bank Blood," *J. A. M. A.* 112: 309, 1939.
14. Ziegler, E. R., Osterburg, A. E., and Horrig, M.: Prothrombin Changes in Banked Blood, *J. A. M. A.* 114: 1341, 1940.
15. Quick, A. J.: The Prothrombin in Preserved Blood, *J. A. M. A.* 114: 1342, 1940.
16. Strumia, M. M., Wagner, J. A., and Monaghan, J. F.: The Use of Citrated Plasma in the Treatment of Secondary Shock, *J. A. M. A.* 114: 1337, 1940.
17. Knott, F. A., and Koerner, E. H.: Storage of Transfusion Plasma, *Lancet* 2: 1069, 1939.
18. Kolmer, J. A., and Howard, M.: Studies on the Preservation of Human Blood, *Am. J. Med. Sc.* 200: 311, 1940.
19. MacDonald, A., and Stephen, G. M.: Changes in Stored Blood, *Lancet* 2: 1169, 1939.
20. Boland, C. R., Craig, N. S., and Jacobs, A. L.: Collection and Transfusion of Preserved Blood, *Lancet* 1: 388, 1939.

THE RETICULO-ENDOTHELIAL SYSTEM: ITS PHYSIOLOGY AND PATHOLOGY*

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IMMUNITY, as currently conceived in the light of the factual evidence accumulated over the past fifty years, consists in the effective interaction of at least two essential components, the one cellular, the other humoral. With the development of vital dyes by Ehrlich,¹ and the demonstration by Goldmann² of their selective segregation in large phagocytic cells throughout the connective tissues, the foundation was laid for studying the protective role of specialized mammalian cells in the disposition of foreign particulate material, both animate³ and inanimate.⁴ The recent chemical synthesis of azo dye antigens⁵ has now provided histo-immunologists with a crucial link in the chain of objective evidence⁶ required to identify this same system of phagocytic cells as the most probable source of circulating, humoral, immune, antibody globulins.⁷ The really tremendous significance of these cellular constituents to mammalian health and survival, therefore, has been gradually but certainly established. Conversely, the physiologic conservational phagocytic activity of this system of cells may become excessive for one or another of the normal circulating elements, resulting in a variety of constitutional pathologic states, which will be analyzed briefly later. Thus, throughout the modern era of the microscopic study of cellular systems and tissue reactions, which has paralleled closely the evolution of the science of bacterial immunology, *functional* rather than purely morphologic considerations have dominated all researches in this field by necessity as much as by choice.

MORPHOLOGIC AND PHYSIOLOGIC CONSIDERATIONS

The speculative curiosity and academic interest of the embryologist and the histopathologist, nevertheless, through the years have continued to guarantee the accumulation of various morphologic details bearing upon the respective origins and the relationships which the phagocytic cells of the blood and tissues bear to each other, and to the nonphagocytic tissue elements. Suffice it to say here, without reviewing in extenso the variety of opinions and evidence presented elsewhere,^{8, 9} that Aschoff and Kiyono¹⁰ in 1913 very well indicated, and at the same time effectively arbitrated, the two chief divergent hypotheses in the conception and creation of the term "reticulo-endothelial system." By this designation it was implied that the definitive phagocytes of blood and tissues, other than the "microphages" (neutrophilic granulocytes) of Metchnikoff,¹¹ could be derived either from connective tissue reticulum or from specialized endothelial cells, or from both, depending upon location and circumstance, and, perhaps, definition. At the present time only minor modifications or elaborations of this broad func-

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tional generalization may be said to have resulted from the intensive and diversified clinical and experimental investigations carried forward during the intervening years.

Any careful, firsthand, objective study of the problem today with the variety of techniques available will reveal certain, readily verifiable morphologic facts, subject to few exceptions, but susceptible of no little controversial interpretation. Reference to Chart I will suggest a skeleton outline of possible cell relationships within the broader limits of the reticulo-endothelial concept. It reflects one more attempt to "streamline" the consensus of current opinion as the basis for this part of our discussion. At the apex is placed the non-controversial, universally accepted, multipotential, mesenchymal anlagen for all blood and connective tissue elements, from which point of departure all cell relationships and interrelationships and interpretations under consideration must by common consent originate. In the most primitive connective tissues at least three morphologic types of cells may always be differentiated: fat cells, endothelial cells, and reticulum cells.

ROLE OF THE FAT CELL

The lipoblast, forerunner of the widely distributed fat cells of the tissues, arises from undifferentiated perivascular, mesenchymal cells related to the reticulum and entirely distinct from fibroblasts (Maximow, Mallory, Friedrich, Wassermann).

Wells¹² has recently emphasized again the necessity of including adipose tissue within the reticulo-endothelial concept in harmony with the glandlike histologic character of the unfatted fat organs, and in conformity with the evidence that many cells distended with fat are still entirely capable of carrying on other functions. Dogliotti¹³ has shown that both brown and white fat tissue cells may store vital dyes like other reticulo-endothelial derivatives, which process is especially well seen in the depleted fat cells; and Bremer¹⁴ has demonstrated in the thin cytoplasmic ring in the periphery of fat-distended cells, granules of vital dyes suggesting participation by fat tissue in antibody formation. As might be anticipated, the opposite interpretation of such objective observations has been advanced, Chun Chang¹⁵ believing his experimentally altered omental and marrow fat cells in the rabbit to be nothing more or less than macrophages with a predominant fat content. He cites the finding of phagocytized hemosiderin and entire granulocytes in such fat-laden cells as circumstantial evidence of a macrophage origin for all fat cells. Certain it is that monocyte and lipoid-stimulated epithelioid cells of blood and tissues frequently show a special type of progressive fatty metamorphosis, resulting in ultimate cell rupture in tuberculosis associated with caseation.¹⁶ Hausberger¹⁷ has reported the ability of the interscapular fat organs to form fat from carbohydrate by way of glycogen deposit in the fat cells. Sehoenheimer's studies¹⁸ with fat marked with heavy hydrogen showed immediate deposition of most of the food fat in specific adipose tissue, rather than direct metabolic utilization. The capacity of depleted fat cells to take up water, thus becoming extremely hydropic, has been noted in our own studies of experimental marrow hypoplasia in pigeons,¹⁹ and observations by Wassermann²⁰ of hydrosis of distended

fat cells led him to suggest that adipose tissue may play an important role in water metabolism. The truth probably lies somewhere between the extremes of the positions taken by the various investigators; viz., that lipoblasts are more or less differentiated cells of the connective tissues, predominantly equipped to efficiently store and promptly release essential fatty substances in the normal metabolic economy of the mammalian body, and on occasion, under special circumstances, such cells may take in small amounts of other substances; the true macrophage, conversely, participates, if at all, in fat phagocytosis only incidental to fat necrosis and to other pathologic disturbances involving fat metabolism, as for example the xanthomatoses.

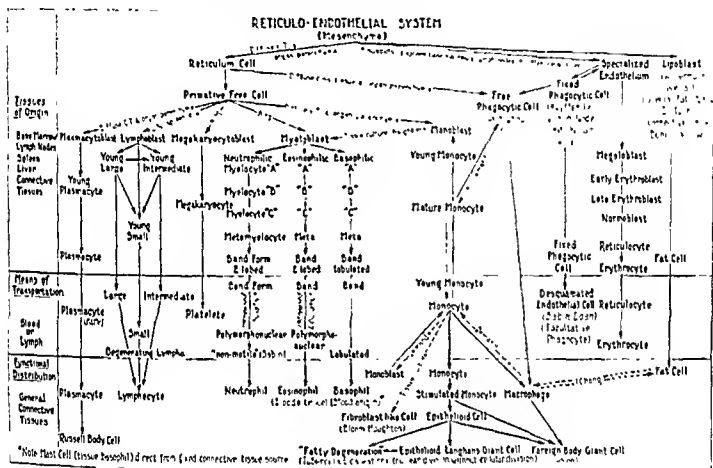


Chart 1.

TISSUE MACROPHAGE

The large highly phagocytic macrophage found in the parenchyma of spleen and bone marrow and scattered throughout the diffuse connective tissues is rarely seen in the circulating blood due to its size and sluggish motility. Such cells divide, giving rise to new, potentially phagocytic units on demand, their ultimate, fixed tissue origin being difficult to determine, being possibly reticulum (Chart 1). They phagocytize large particles of debris, digest whole red and white blood cells, selectively segregate vital dyes, and show intravacuolar reactions to neutral red, with a pH range from 6.8 to 8. Anchored in the sinuses of the liver and presumably deriving from the lining endothelium are functionally and morphologically similar units, the so-called Kupffer cells. In lymph nodes subject to drainage from areas of necrosis or hemorrhage, the sinus endothelium hypertrophies, becomes highly phagocytic, and individual cells may even detach themselves, becoming free and rounded phagocytes.

THE MONOCYTE

On the other hand, always to be found in the circulating blood physiologically are the potentially phagocytic monocytes (large mononuclear or transitional cells of Ehrlich), which when stained supravitaly with neutral red, reveal a more or less definite rosette pattern of small preformed segregation vacuoles, with constant pH.²¹ Their number in the blood is usually a direct reflection of their quantitative, extravascular representation in organs and tissues of the body, and they participate more or less actively in the cellular reaction to a variety of stimuli.²² These blood monocytes appear to arise in the intervascular tissue spaces in association with undifferentiated, fixed reticulum cell hyperplasia, and must undergo a period of development with the elaboration of characteristic mitochondria and specific vacuoles before motility or functional maturity are attained. However, under stress, when engorged with whole blood cells or excessive nonspecific debris, the definitive phagocytes in a given inflammatory area frequently become indistinguishable morphologically in terms of their endothelial or reticular cell origin. The term "macrophage" (big eater) correctly and accurately describes such cells irrespective of their remote derivation.

INTERRELATIONSHIPS OF THE PHAGOCYTIC CELLS OF MAMMALIAN TISSUES

Starting, therefore, ultimately either from specialized fixed endothelium or from fixed reticulum cells with primitive mesenchymal potencies, certain free phagocytic cells arise, showing more or less differentiating criteria with respect to cell size, morphology, staining reaction, degree of motility, intravacuolar pH, segregation capacity, and selectivity. Under various pathologic conditions and during *in vitro* cultivation, these differences may be either accentuated or minimized. Such firmly established and well-documented observations have resulted in two principal divergent interpretations; first, that all morphologic variations reflect simply different phases in the life cycle and/or stage of functional activity of one cell strain; second, that environmental circumstances more or less modify or efface the original identifying criteria of cells with phagocytic potencies arising from two or more sources.

The evidence adduced to date from tissue culture has tended largely to support the first conception. In the earlier studies the survival *in vitro* of living blood or tissue cells was associated almost invariably with extreme vacuolization, much of which was degenerative. With improvements in technique, more particularly the determination of essential nutritional elements and optimum gas concentrations for explanted cells, and with adequate provision for elimination of catabolic products, conditions were rendered more nearly physiologic and interpretations have been correspondingly more readily transferable to *in vivo* phenomena. During recent years in this laboratory, Houghton²³ has directed *in vitro* culture studies toward the better understanding of this problem of the interrelationship of blood and tissue phagocytic cells. The monocyte of the normal blood would seem from these observations to have very considerable powers of survival, multiplication, and adaptation to functional requirements, more certainly than can be demonstrated for circulating lymphocyte, or for any of the granulocytic series of cells. Under these con-

ditions definitive "macrophages" appear, which are indistinguishable from the tissue macrophages and which quite obviously have been derived from the circulating blood monocytes.

Ebert and Florey²⁴ modified the Sandison-Clark chamber technique for the *in vivo* study of the extravascular development of the monocyte in the rabbit's ear. Tissue cells were marked with vital new red, pontamine sky blue, or trypan blue. With the aid of carbon injections, circulating ink-containing "monocytes" were observed to emigrate into the organizing clot on the growing edge of an injured tissue area. However, "though it was thus made certain that the blood monocyte was capable of developing extravascularly into a cell indistinguishable from that frequently called a histiocyte, it was not quite clear that *all* the macrophages present at the developing edge originated from monocytes. It is conceivable that some were due to the mobilization and division of pre-existing tissue histiocytes." While supravital preparations of rabbits' blood stained with neutral red were reported to have been made, no study of the tissue cells under similar conditions is recorded by these investigators. My co-workers and I^{16, 21} have shown that acid diazo dyes in dosages comparable to those used by Ebert and Florey, while prominently identifying the so-called macrophages or histiocytes already in the tissues, are only rarely, if at all, demonstrable within the monocytic derivatives, if the latter are defined and identified by the rosette of vacuoles, revealed by superimposing vital neutral red upon the living tissues in question. Ebert and Florey interpret their data as supporting the monocytic origin of the tissue histiocyte, but in all fairness, a critical review of their evidence is hardly more convincing or conclusive in that direction than many previous studies. Of genuine interest, from the functional standpoint, however, are their observations suggesting that macrophage-ingested erythrocytes may be completely broken down within two hours, and that these same tissue macrophages, containing ingested vaseline, survived, and remained relatively stationary in position and condition within their rabbit ear "chambers" over an eleven-month period.

Reference to Chart 1 again may assist in clarifying the ramifications and probable relationships of those morphologically differentiable cells which contribute ultimately to the group of functionally active phagocytes known descriptively and collectively as macrophages. From fixed endothelium and from fixed reticulum—either directly, or via the lipoblast-fat cell route, or through the monoblast-monocyte-epithelioid maturational development—may be derived definitive units, which become increasingly difficult to recognize in terms of their origin, as maximum, heterogeneous, nonspecific, phagocytic activity obscures all other cytoplasmic criteria. Under physiologic conditions there can be no doubt as to their individual identity and more or less selective functions: the tissue-distributed macrophages of spleen and bone marrow always contain senile red blood cells in their role of conservator of iron to the body, and an occasional identifiable white cell; the Kupffer cells of the liver and the mesenteric lymph node endothelium contain debris salvaged from blood and lymph streams in the normal course of keeping the blood stream free of bacteria and of partially broken down proteins from the gastrointestinal tract; the monocyte retains its rosette of vacuoles, and is fastidious in its selection of only small particulate

debris in contradistinction to the tissue elasmatoocyte or macrophage; and the fat cells clearly limit their activities to the storage of lipoids. True, however, to the universal biologic principle of a common responsibility for community survival when external danger threatens, any or all of these physiologically distinctive units may, under pathologic conditions, become mobilized in a common cause involving the utilization of a common potential phagocytic capacity, and, to a greater or lesser degree, each may be able to contribute to the humoral antibody globulins so essential to adequate cellular defenses. In this connection, Sabin⁶ has pointed out that even the fibroblast may be seen to take up minute amounts of vital dyes or dye antigens when excessive quantities are forced upon the tissues, and that they may contribute their share to the circulating humoral antibodies.

Just as these various cells function more or less selectively in response to specific physiologic demands, thereby assuming certain distinguishing morphologic characteristics, it seems reasonable to assume that under differing pathologic requirements one or other of these units might be able to assume the major role of defense, to be secondarily aided from other areas, if and whenever necessary. Such has been demonstrated to be the case. In tuberculosis the monocyte-epithelioid response appears to be primary;¹⁶ in kala-azar the macrophagic ingestion of *Leishmania donovani* bodies leaves the monocytes of the hamsters relatively unaffected.²⁵ As further studies of pathologic reactions are analyzed on the basis of these cellular concepts, the examples will doubtless be multiplied.

Turning now to other areas where the interrelationships are perhaps less involved, we come to the question of monoblast and myeloblast. Usually under both physiologic and pathologic conditions these stem cells, precursors of their respective cell strains, may be more or less readily differentiated. The criteria are admittedly more readily applied in supravital preparations and Romanowsky stained blood films than in fixed sections. It is in tissue culture, however, that the potential capacities for dedifferentiation have been most strikingly observed. Houghton²³ believes, from his studies of normal human blood, that the myelocytes which appear in his cultures after three to six weeks are not arising from "primitive cells," but from the so-called "monocytes," which gradually lose their more mature characteristics and then begin to elaborate specific granules or to develop vacuoles and phagocytic capacities typical of the macrophage. In cultures of leucemic monocytes there is the uniform appearance of fibroblast-like cells after a few days, unquestionably derived from the monocytic elements; this is in sharp contrast to cultures of lymphatic and myeloid leucemic cells. Houghton's observations would tend to invest the monocyte with the most versatile capacities for response to environmental demands of any of the blood or tissue elements. Aside from this unexpected attribute of specific granule elaboration by the monocyte in cultures, the myeloblast of the normal bone marrow differentiates from parenchymal reticulum cells, develops specific granules, either neutrophilic, eosinophilic, or basophilic in reaction to the Romanowsky stains, and follows the maturational sequence generally recognized.¹⁹

The lymphocyte, with its lack of differentiating characteristics, continues to intrigue the imagination of all cytologists as the most logical "primitive

cell" from which an elaboration of specific cytoplasmic structures or materials may arise. However, as increasingly detailed and careful cytologic and functional studies are applied to lymphocytes in blood, lymph, lymph nodes, and tissues, it becomes ever more evident that this cell has an independent life cycle²⁵ and existence (Chart 1) altogether comparable with those observed for the myeloid and erythroid elements.¹⁹

The plasma cell, while always found in close association with the lymphocyte, physiologically and in chronic inflammatory tissue reactions, would seem to stem back rather directly to the primitive free cell arising from reticulum, and to develop from an immature plasmacytoblast with highly individual mitochondrial characteristics rather than from the differentiated lymphocyte.

The intravasular origin of megaloblast and red blood cell series from fixed, specialized, hematogenic, capillary endothelium in bone marrow²⁷ is indicated on Chart 1 for the sake of completeness, and to suggest the functional relationships of endothelium under differing circumstances, but has no other place in this discussion.

PATHOLOGIC CONSIDERATIONS

Congenital Hemolytic Jaundice.—Directly related to the well-established physiologic phagocytic function of the reticulo-endothelial system for senile, or damaged, or imperfect blood cells would seem to be certain constitutional pathologic states in which an excessive destruction of these same elements is at least one factor contributing to the clinical symptom syndromes. Normal erythrophagocytosis, occurring in the macrophages of spleen, liver, and bone marrow in varying degree in different species, is followed promptly by red blood cell disintegration and by a splitting of the hemoglobin molecule into globin and heme. The latter is further broken down into (1) the iron-free pigment *hematoidin*, which becomes the bilirubin of the plasma in hemolytic jaundice, or is secreted by the hepatic parenchymal cells as bile; and (2) *hemosiderin*, in which form iron remains stored until needed for hemoglobin re-synthesis in the bone marrow. Mann and his associates²⁸ have demonstrated conclusively that the macrophages of spleen and bone marrow, not the stellate cells of the liver, are normally the principal sources of bilirubin, and Kanner²⁹ has reported, and we can confirm, that no histologic alteration in the Kupffer cells of the liver occurs in hemato-genous icterus. Upon these observations may be based the modern approach to the pathologic physiology of human congenital hemolytic icterus. A dominantly inherited constitutional tendency to excessive hemolysis, upon which many environmental factors (pregnancy, trauma, minor infections) may play disastrously, has been clearly established.³⁰ The more active phases of this disease are invariably associated with a directly proportionate degree of splenomegaly, in which excessive parenchymal erythrocyte segregation and phagocytosis may readily be demonstrated. Moreover, splenectomy, when successfully accomplished, and if it includes the removal of all accessory splenic tissue—even though the operation be performed during an acute hemolytic crisis³¹—invariably results in an immediate, complete, and usually permanent remission. Any significance, other than diagnostic, of defective, spherocytic erythrocytogenesis in congenital hemolytic icterus remains unsettled. Haden³² believes spherocytosis to be the congenital prerequisite to increased erythrocyte

fragility, while Dameshek³³ interprets spherocytosis as the end result of hemolysin activity upon mature red blood cells without any inherent marrow defect. The fact that splenectomy is followed by a return not only of red blood cells, but also frequently of thrombocytes and granulocytes, to more nearly normal, highly stable, equilibria, at least suggests that any marrow contribution to this clinical syndrome is dependent upon a more fundamental splenic dysfunction. Farrar, Burnett, and Steigman³⁴ report a patient with hemolytic anemia with hepatic degeneration who responded dramatically to emergency splenectomy although excessive erythrophagocytosis by splenic macrophages was not apparent. An hemolysin was demonstrated in the blood serum before, but not after, removal of the spleen. Thus, at least two possible mechanisms of red blood cell destruction must be attributed to the spleen in hemolytic states; the one, direct phagocytosis by reticulo-endothelial cells, the other, indirect hemolysin elaboration, probably by the same cells.

Thrombocytopenic Purpura.—Reference has been made to the frequency with which a mild thrombocytopenia and granulocytopenia are found secondary to primary splenic anemia.³¹ Either of the former disequilibria may become primary in certain individuals and dominate the clinical and laboratory pictures. There is one type of thrombocytopenic purpura directly related to a normal-sized but pathologic spleen, and dependent upon, either selective abnormal macrophagic destruction of platelets³⁵ or splenic inhibition of marrow megakaryocytic functional efficiency,³⁶ or "thrombocytopen"³⁷ derived from the spleen. In these patients, successful splenectomy is followed by a prompt and maintained return of platelets to the circulation without any recognizable change in number and histologic integrity of marrow megakaryocytes before and after surgery.³⁸ The reticulo-endothelial phagocytes in these spleens probably play a more or less important role in the peripheral destruction of the important platelet elements.

Primary Splenic Granulocytopenia.—Recently a primary granulocytopenic syndrome, with marrow myeloid hyperplasia, splenomegaly without hepatic pathology, and a negative history for drug or bacterial idiosyncrasies, has been described from this clinic.³⁹ Hemolytic erythrocytopenia and/or thrombocytopenia have been either incidental or nonexistent. The clinical manifestations have ranged from acute, through subacute, to chronic, and in each instance splenectomy has resulted in complete recovery. Careful supravital surveys have confirmed in every instance a tremendous excess of highly phagocytic macrophages in the splenic parenchyma, with whole intact granulocytes representing the principal engulfed material; the organs, fixed and sectioned, using ordinary techniques, lost much of the dramatic demonstration of selective phagocytosis seen in the fresh tissues.

Banti's Syndrome.—In Banti's syndrome the splenomegaly found accompanying progressive hepatic cirrhosis of varying etiology⁴⁰ is characteristically associated with a granulopenic leucopenia, which is partially relieved by splenic artery ligation, and completely corrected by splenectomy.⁴¹ Here again the sternal marrow shows no interference with normal hematopoietic activity, and any anemia is usually secondary to occult or obvious gastrointestinal hemor-

rbages. Both leucopenia and moderate thrombocytopenia, on the contrary, are apparently related to an hyperplasia of overactive macrophages in an hypertrophied spleen.

The evidence just presented, viewed in its entirety and in its interlocking and overlapping details, would certainly seem to justify the belief that the physiologic function of reticulo-endothelial phagocytosis by the normal spleen may become pathologically accentuated, either as an inherited or as an acquired trait; and that any or all of the elements arising in the marrow physiologically may be individually or collectively preyed upon to the extent or degree of producing symptoms characteristic of each critical deficit point, respectively. In addition to the usual information, differential and quantitative cell studies of the sternal marrow, together with data obtainable from the adrenalin test, are essential to the critical analysis of the mechanism underlying these several syndromes, and as a basis for determining their therapeutic rationale.

RELATIONSHIP OF THE RETICULO-ENDOTHELIAL SYSTEM TO HUMORAL IMMUNITY

In addition to the observations of selective phagocytic activity on the part of the reticulo-endothelial cells, a great many investigations have been undertaken in the attempt to demonstrate a direct relationship between this cellular system and humoral immunity. Reticulo-endothelial cell blockade with carbon particles or various vital dyes, or extirpation of the spleen, or both, have preceded the administration of a variety of antigens, with the variation in antibody titers, subsequently developed, used to measure the specific sensitivity of the tissue response. Most of the evidence thus accumulated has seemed to indicate the existence of an intimate relationship between the integrity of the reticulo-endothelial system and antibody formation.

When foreign erythrocytes are injected into an animal for the first time, they are promptly removed and destroyed by intracellular phagocytosis; when red blood cells from the same source are again injected after a suitable interval, intravascular, extracellular hemolysis occurs through the action of circulating hemolysins. That the cellular elements originally responsible for the elimination of the foreign red blood cells by phagocytosis should be the most likely source of the newly developed hemolysins is a reasonable assumption. In support of this hypothesis is the almost complete agreement that hemolysin production is interfered with to a greater or lesser degree by reticulo-endothelial blockade.⁴²

Persistent endocrine therapy has been frequently observed to lead to the development of hormone refractoriness. Collip and his collaborators⁴³ have shown that acquired hormone refractoriness is serologically transferable, and certain studies have pointed toward a compensatory hypertrophy of antagonistic endocrine glands as the underlying mechanism.⁴⁴ The extensive and highly suggestive experimental studies reported by Gordon, Kleinberg, and Charipper,⁴⁵ however, seem to link the reticulo-endothelial system to this phenomenon. They found that, while the daily subcutaneous injection of pregnant mare's serum, gonadotropin, caused a maximum ovarian weight increase in normal female rats by the seventeenth day, in parallel tests with splenec-

tomized female rats the initial rate of ovarian increase was twice as fast, and the development of refractoriness was delayed until the twenty-fifth day. By combining splenectomy with trypan blue blockade, hormone refractoriness with resulting regression in ovarian weight was not observed even after fifty-five daily hormone injections. Refractoriness to thyrotropic hormone in normal guinea pigs developed within twenty days, whereas in splenectomized, dye-blockade controls it was delayed for at least fifty days. The effects of splenectomy on the development of testicular hypertrophy in male rats following daily injections of gonadotrophic substance were in accordance with the same law. Gordon and his colleagues argue that since participation of the reticulo-endothelial system seems to be essential for the development of hormone refractoriness, antihormones are true antibody, immunologic adaptations despite their alleged failure to give routine, complement deviating, test tube reactions.

Most convincing, however, in this general area have been the recent cooperative investigations of chemist, immunologist, and cytologist, each utilizing the latest technical approaches to the problem in his own domain. In 1930 Heidelberger and Kendall⁵ synthesized a dark red dye protein, R-salt-azobenzidine-azo-egg albumen, with marked antigenic properties, that showed highly selective phagocytosis by cells of the reticulo-endothelial system.

Preparing a more highly purified antibody than had previously been available, Heidelberger and his associates,⁷ using the ultracentrifuge and determining electrophoretic mobilities, found the molecular weights and electrical phenomena of antibodies to be those characteristic of proteins. This was in conformity with the previously observed invariable association of antibodies with the globulin fractions in serum. Sabin,⁴⁶ it will be remembered, pointed out in her early study of the chick blastoderm, among other things, the liquefaction of some of the angioblastic cells differentiating from the primitive mesenchyme to form the first protein-containing blood plasma. Ranvier⁴⁷ had noted the "shedding of exoplasm" by certain of the larger connective tissue cells in the frog, which led him to designate these elements as clasmatocytes. Supravital preparations of living cells from blood, lymph nodes, spleen, omentum, or peritoneal fluid regularly confirm the fragmentation of cytoplasm. This phenomenon is especially prominent in young lymphocytes, monocytes, and clasmatocytes or macrophages. These observations suggest the mesothelial origin of some, if not all, of the serum proteins; and the increase in fibrinogen and globulin following large doses of India ink and saccharated iron oxide⁸ has been explained on the basis of a stimulation of the tissue phagocytes. Recently Sabin⁶ took the alum precipitate of Heidelberger's dye protein, whose readily visible purplish-red particles had been proved to be of superior antigenic potency,⁷ and followed its physical disposition via various avenues of injection in rabbits. When given intravenously, the resulting antibody titer was higher than when the antigen was introduced by the intradermal, subcutaneous, or intraperitoneal routes, with phagocytosis by local macrophages and by the lymphatic endothelium and free macrophages of the regional lymph nodes only.

The details of this study make fascinating reading, but the evidence as finally interpreted seemed to justify the following conception: the phagocytic endothelium, both vascular and lymphatic, as well as the free macrophages

of the tissues everywhere, are constantly phagocytizing foreign materials which may be divided into two general groups, antigens and nonantigens. These substances are first segregated into the vacuolar organs of digestion of the cell and prepared for diffusion into the cytoplasmic zone of synthesis; the normal food substances ingested are utilized in the formation of normal globulin, but foreign antigenic proteins increase and modify this synthesis of globulin, so that with the shedding of parts of the surface films of such cells, both normal and antibody globulins are carried into the blood plasma. This conforms with the observation of a concomitant increase in both under such circumstances. These cells, during the period in which the modified globulin remains within the cytoplasm, react differently from the normal cell in the presence of additional original antigen, thus relating "sensitization" and "immunization" to different phases of the same mechanism.

The variation in species and individual response to various antigenic substances is readily explained by Sabin's observations. Two distinct steps are necessary for the development of antibodies: first, the phagocytosis and intravacuolar preparation of the antigen for introduction into the cytoplasm proper; and, second, the synthesis of new and modified globulins. Both these phases require time, which would naturally vary with the quantity of antigen available, its relative ease of degradation, and its ability to modify normal globulin synthesis. Furthermore, the units which comprise this phagocytic defense system of the mammalian organism are as diversified in origin and definitive type, and probably, therefore, in functional specificity and efficiency, as are the several units of a modern arm—granulocytes, monocytes, mastocytes, endothelial phagocytes, specialized as in the Kupffer cell and nonspecialized as in the lining cells of the lymph sinuses; and it is probable that each responds differently, quantitatively if not qualitatively, to different antigens and under different environmental circumstances. The various chemotherapeutic agents either assist or prevent clinical recovery in infectious diseases to the extent that they aid or hinder this important cellular mechanism of the body in its phagocytic and antibody-forming functions plus any direct effect they may have on pathogenic bacteria.

These recent studies of Dr. Sabin and her chemical and immunologic collaborators mark an enormous advance in our understanding of the essential mechanism of natural and acquired immunity, yet represent but a logical consummation and final unique demonstration of an hypothesis supported by the gradually accumulated experience of many years.

SUMMARY

In summary, the following points may be accepted as a tentative working basis for present therapeutic applications, and for further exploratory excursions into this absorbing and important phase of medicine: (1) the reticulo-endothelial system represents a functional unit arising from mesenchymal derivatives possessing a common phagocytic capacity; (2) certain morphologic variations associated with a variety of physiologic functional requirements may be recognized; (3) under pathologic conditions and in tissue culture such morphologic criteria may become either accentuated or diminished; (4) when highly stimulated, all phagocytic cells, regardless of the ease of differential

identification under physiologic conditions, tend to assume a common appearance and may be designated by the common descriptive term, macrophage; (5) while originally protective and conservational in functional objectives in the normal body economy, these phagocytic scavengers of the mammalian tissues may assume an excessive selective destructive affinity for any of the normal blood elements, thus precipitating a variety of characteristic clinical syndromes; (6) the spleen is usually the site of the greatest destructive activity, and when the appropriate diagnostic tests have localized the major pathology to this organ, splenectomy is indicated as the rational therapeutic procedure; (7) conclusive evidence of the active participation of the reticulo-endothelial cells in humoral antibody production would now seem to have been obtained; (8) this reaction has both favorable (protective antibodies) and unfavorable (hemolysin production) implications for mammalian survival; (9) a constantly increasing accumulation of carefully controlled factual information is providing an ever broader and more substantial foundation for a more effective manipulation of this important defense unit in the interest of individual health.

REFERENCES

1. Ehrlich, P.: *Farben analytische Untersuchungen zur Histologie und Klinik des Blutes*, Berlin, 1891, August Hirschwald.
2. Goldmann, E. E.: *Die äussere und innere Sekretion des gesunden Organismus im Lichte der "vitalen Färbung,"* Tübingen, 1909, H. Laupp.
3. Wyssokowitsch: *Ztschr. f. Hyg. u. Infektionskrankh.* 31, 1886.
4. Evans, H. M., and Seott, K. J.: *Contribution to Embryology*, Carnegie Institute, Washington, D. C. 10: 1, 1921.
5. Heidelberger, M., and Kendall, F. E.: *J. Exper. Med.* 58: 137, 1933; 59: 519, 1934; 62: 467, 1935.
6. Sabin, F. R.: *J. Exper. Med.* 70: 67, 1939.
7. Heidelberger, M., and Pedersen, K. O.: *J. Exper. Med.* 65: 393, 1937.
8. Jaffe, R. H.: *Downey's Handbook of Hematology*, New York, 1938, Paul B. Hoeber, Vol. 3, Chapter 15.
9. Doan, C. A.: *The Reticulo-endothelial System. International Symposium on the Blood*, Madison, 1939, Univ. Wis. Press, p. 167.
10. Aschoff, L., and Kiyono, K.: *Folia haemat.* 15: 383, 1913.
11. Metchnikoff, Eli: *Pathologie comparée de l'inflammation*, Paris, 1892.
12. Wells, H. G.: *Adipose Tissue a Neglected Subject*, J. A. M. A. 114: 2177, 2284, 1940.
13. Dogliotti, G. C.: *Ztschr. f. Zellforsch. u. mikr. Anat.* 8: 222, 1928.
14. Bremér, J. L.: *Anat. Rec.* 70: 263, 1938.
15. Chang, Chun: *Anat. Rec.* 77: 397, 1940.
16. Sabin, F. R., Doan, C. A., and Forkner, C. F.: *Studies in Tuberculosis*, *J. Exper. Med. Supp.* No. 3, 52: 1-152, 1930.
17. Hausberger, F. X., and Gujot, O.: *Arch. f. exper. Path. u. Pharmacol.* 187: 647, 1935.
18. Schonheimer, R., and Rittenberg, D.: *J. Biol. Chem.* 3: 163, 1935.
19. Doan, C. A.: *Clinical Implications of Modern Physiologic Hematology*, Beaumont Foundation Lectures, St. Paul, 1936, Bruce Publishing Co.
20. Wassermann, F.: *Sitzber. Ges. Morph. München* 42: Nov. 7, 1933.
21. Sabin, F. R., Doan, C. A., and Cunningham, R. S.: *Contribution to Embryology*, Carnegie Institute, Washington, D. C. 16: 125, 1925.
22. Doan, C. A., and Wiseman, B. K.: *Ann. Int. Med.* 8: 383, 1934.
23. Houghton, Ben C.: Data in preparation for publication.
24. Ebert, R. H., and Florey, H. W.: *Brit. J. Exper. Path.* 20: 342, 1939.
25. Meleney, H. E.: *Am. J. Path.* 1: 147, 1925.
26. Wiseman, B. K.: *J. Exper. Med.* 54: 271, 1931; *Folia haemat.* 46: 346, 1932.
27. Doan, C. A., Cunningham, R. S., and Sabin, F. R.: *Contribution to Embryology*, Carnegie Institute, Washington, D. C. 16: 163, 1925.
28. Mann, F. C., Bollman, J. L., and Magath, T. B.: *Am. J. Physiol.* 69: 393, 1924.
29. Mann, F. C., and Bollman, J. L.: *Arch. Path. & Lab. Med.* 2: 516, 1926.
30. Kanner, O.: *Compt. rend. Soc. de biol.* 95: 1311, 1926.
30. Doan, C. A., Wiseman, B. K., and Erf, L. A.: *Ohio State M. J.* 30: 493, 1934.

31. Doan, C. A., Curtis, G. M., and Wiseman, B. K.: *J. A. M. A.* 105: 1567, 1935.
32. Haden, R. L.: *Am. J. M. Sc.* 188: 411, 1934.
33. Dameshek, W., and Schwartz, S. O.: *Medicine* 19: 231, 1940.
34. Farrar, G. E., Burnett, W. E., and Steigmann, A. J.: *Am. J. M. Sc.* 200: 164, 1940.
35. Kaznelson, P.: *Wien. klin. Wchenschr.* 29: 1451, 1916; *Ztschr. f. klin. Med.* 87: 133, 1919; *Wien. Arch. f. inn. Med.* 7: 87, 1923.
36. Frank, E.: *Bull. klin. Wchenschr.* 52: 454, 490, 961, 1062, 1915.
37. Troland, C. L., and Lee, F. C.: *J. A. M. A.* 111: 221, 1938.
38. Wiseman, B. K., Doan, C. A., and Wilson, S.: *J. A. M. A.* 115: 8-13, 1940.
39. Wiseman, B. K., and Doan, C. A.: *J. Clin. Investigation* 18: 473, 1939.
40. Rousselot, L. M.: *Bull. New York Acad. Med.* 15: 188, 1939.
41. Doan, C. A.: On the Origin and Developmental Potentialities of Blood Cells, *Bull. New York Acad. Med.* 15: 668, 1939.
42. Cannon, P. R., Buer, R. B., Sullivan, F. L., and Webster, J. R.: *J. Immunol.* 17: 441, 1929.
43. Collip, J. B., Selye, H., and Willimsson, J. L.: *Endocrinology* 23: 279, 1938.
44. Sulman, I.: *J. Exper. Med.* 65: 1, 1937.
45. Gordon, A. S., Kleinberg, W., and Charipper, H. A.: *J. Exper. Med.* 70: 333, 1939.
46. Sabin, I. R.: Contribution to Embryology, Carnegie Institute, Washington, D. C. 9: 213, 1920.
47. Ranvier, L.: *Arch. d'nnat. micr.* 3: 123, 1900.

THE MECHANISM OF BACTERIAL ALLERGY*

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WHENEVER antigenic substances of foreign origin are brought into contact with inner tissues of animals past the natural barriers, the physiologic behavior of such animals is radically changed: they are rendered allergic (allergy = altered reactivity). In final analysis this alteration consists of an acquisition by the tissues of these animals of a specifically directed increased capacity to immobilize, neutralize, and eventually to eliminate the particular foreign matter if it is subsequently introduced.

While different antigens vary as to their effectiveness in causing the establishment of this altered reactivity, in general the necessary stimulus for altering the reactivity of the exposed tissues (immunogenic = allergenic stimulus) can be produced by the parenteral entry of extremely small amounts of antigens (allergens). Thus, even in the case of antigens possessing an intrinsic toxicity or a capacity to engender infection, this alteration of reactivity can be accomplished by introducing these antigens in amounts below the quanta necessary to cause primary injury (though in practice it is preferable to use for this purpose larger amounts of modified material rendered atoxic or avirulent).

The specificity of this altered response is mediated by antibodies which thus may serve as indices of the past immunogenic (allergenic) experience of a given animal. If the antibodies are produced in sufficient abundance to appear free in the circulation, the blood serum of such animals may serve for a passive transfer of this specific altered reactivity to normal animals.

Besides noting the presence of antibodies in the blood or tissue juices, the state of specifically altered reactivity of the animal can be detected either by observing the response of the animal as a whole (systemic reaction) to systemic reintroduction of the antigen in question, the local inflammatory reaction produced in certain most accessible tissues by the exposure *in situ* (skin, conjunctiva, nasal mucosa), or by exposing excised reactive tissues to contact with antigen *in vitro* (Schultz-Dale reaction).

While the term "allergy" was coined by von Pirquet¹ to denote the totality of this specifically altered reactivity, irrespective of the method by which it is demonstrated, and disregarding the character of the final effect produced in the animal, in current colloquial usage the term "allergy" has been frequently employed more specifically with reference to the inflammatory skin response elicited in previously exposed (immunized = allergic = sensitized) animals by intracutaneous introduction of the antigen (allergen) in question (which presumably causes no such reaction in the skin of normal animals). In

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this narrow sense "bacterial allergy" denotes the skin reaction elicited by bacteria and their products. This reaction is utilized in clinical practice to detect the existence of a specific altered reactivity presumably engendered by previous exposure to the particular bacterial antigen.

Because of certain differences in the general characteristics of skin reactions to certain bacterial antigens as compared with response to other antigens such as soluble proteins, it is currently and erroneously assumed that the mechanism of bacterial allergy is essentially different from that of hypersensitiveness to other antigens. This unwarranted assumption is largely due to the fact that much of the early knowledge concerning bacterial allergy came by way of the study of hypersensitiveness in tuberculosis.

The hypersensitiveness developed as a result of exposure to the tubercle bacillus, however, is not entirely typical of bacterial allergy in general. In some respects it represents an exception in that the exposed animal in addition to producing antibodies (and thus becoming sensitized) to preformed antigens present in the bacillus itself (as in the case of an exposure to other bacterial antigens), develops also sensitivity to some as yet not definitely known product originating within the infected tissue (tubercle). While direct evidence for the existence of this product is not abundant, the experiments of Mc Junkin² (with peritoneal exudates of tuberculous animals) and those of Zinsser and Grinnell³ (in which delayed necrotic tuberculin-like reaction was obtained with products of autolyzed pneumococcus in animals previously sensitized with large amounts of this organism) point in this direction. Moreover, the findings of Dienes⁴ clearly show that delayed inflammatory (tuberculin type) response may be elicited with antigens other than bacterial, notably with egg white (and presumably with other common antigens), provided that on the first exposure the antigen is introduced (directly or indirectly) into a site in which an inflammatory process has been set up. In short, the basic mechanism of allergic response in tuberculosis is not different. It represents merely a modification in some of the details of the reaction due to superimposition of the peculiar properties of the tubercle bacillus and of the pathologic process which it engenders; under suitable conditions this modified type of response can be superimposed on the usual allergic reaction to such typical antigen as egg white.

That the response of hypersensitive animals to bacterial antigens is basically identical with that observed with typical antigen derived from other sources has been shown in some of the earliest studies. Thus Krauss and Doerr found in 1908 that intravenous injection of bacterial extracts into guinea pigs causes typical anaphylaxis and death of sensitized animals⁵ and that the serum of animals sensitized with bacterial vaccines is capable of inducing hypersensitiveness in normal animals, so that such passively sensitized animals respond with anaphylactic shock to a subsequent intravenous injection of homologous bacterial extracts.⁶ More recently, Zinsser and Parker⁷ showed that isolated smooth muscle of either actively or passively sensitized guinea pigs responds with typical contraction when it is exposed *in vitro* to contact with specific bacterial extracts.

Even in the case of hypersensitiveness to antigen of tubercle bacillus these basic responses have been elicited, provided that the complicating factors incident to the complexity of the antigenic structure of the bacillus and of the pathologic process it engenders are eliminated. Thus Enders⁸ produced typical anaphylaxis in guinea pigs by intravenous injection of the soluble specific substance (specific hapten) of this organism both in animals actively sensitized with dead tubercle bacilli and in those passively sensitized with immune rabbit serum. Similarly, instead of a delayed necrotic reaction typical of the response in infected animals, an immediate evanescent wheal and erythema, characteristic of the usual response to ordinary soluble antigens, is elicited when animals sensitized with extracts of ground-up tubercle bacilli receive intracutaneous injection of tuberculin.⁹

It is to be noted, however, that in all the experiments already quoted, the antigenic materials used for sensitizing the animals, and particularly for eliciting the typical immediate response of hypersensitive animals, consisted either of crude bacterial extracts or of separate soluble fractions isolated from the latter. When coarse fragments of bacteria or suspensions of intact organisms are used in place of extracts, the local response of sensitized animals consists of an atypical delayed inflammatory reaction; and when such particulate antigens are introduced intravenously, one may fail to elicit any symptoms whatever if all soluble material has been carefully removed by centrifugation. To elicit typical symptoms analogous to those observed with ordinary soluble proteins (such as egg white, serum, etc.) bacterial antigens must also be present in the state of solution. However, this dependence of the symptomatology of allergic response on degree of dispersion of the antigen is not a particular attribute of bacterial allergy. The failure to elicit typical reactions has been noted with any antigen if it is used in a more or less coarsely particulate state, such as erythrocytes, for instance. That the physical state of antigen is the determining factor is particularly clearly seen in the instance of such common antigens as egg white, which similarly fails to elicit characteristic reactions in sensitized animals if the test injection of this antigen is given in the form of coarse particles (as seen when suspension of particles of collodion coated with egg white are injected instead of a solution of egg white). If this question of the degree of dispersion of antigenic material is kept in mind, it becomes clear that atypical reactions observed with bacterial antigens do not indicate any difference in the basic nature of the reaction and do not justify the separation of bacterial allergy as being due to a different mechanism.

Similarly, the claim that bacterial allergy seems to differ from allergy to soluble proteins in that it may be elicited in certain instances in the absence of demonstrable antibodies, is also invalidated upon adequate analysis. It should be noted that intact bacteria in general possess a relatively low antigenic activity. Even in the case of bacterial extracts, several injections of antigen are required for the production of appreciable concentration of circulating antibodies, as well as for securing effective experimental sensitization. This is most likely due to the fact that bacterial substance, even in extracts, is for the most

part *not* in the state of true solution, and a large part of it is phagocytosed, digested, and thus eliminated from the circulation before it has had opportunity to produce widespread antigenic stimulation upon the body cells necessary for the copious production of antihodies.

On the other hand, small amounts of antigen circulating in the blood even for a relatively short time may be fixed by such tissues as the skin, an organ known to take up very quickly from the circulation many (but not all) substances introduced into it,¹⁰ and produce local antigenic stimulation. It is for this reason that not infrequently the skin test to bacterial protein (as well as to other poor antigens) may be positive, while the blood stream may show so low a concentration of antibody that its demonstration by usual serologic methods may fail altogether. Moreover, similar irregularities can be shown to exist under certain conditions even in the case of ordinary protein antigens. For instance, if a normal guinea pig receives a sensitizing injection of egg white or of horse serum and subsequently is tested by daily intradermal injections of the homologous antigen, the character of the response to intradermal injection will vary as the antibodies are being gradually produced. The skin test performed twenty-four hours after the sensitizing injection will be negative; that is, the animal still fails to react allergically—it behaves like any normal animal. However, on the second or on the third day (while no circulating antibody can as yet be demonstrated), the animal may exhibit a modified skin response—namely, at the sight of injection there will appear a wheal. However, instead of appearing immediately after injection as is the rule when soluble protein is injected into the skin of a fully sensitized animal, there will be a delay of from eight to twenty-four hours. Only after several days (five to seven or more) have elapsed after initial sensitization will the animal respond with a typical evanescent wheal and erythema, appearing within a few minutes after intradermal injection of the antigen; at this time circulating antibodies can usually be found. This occasional failure to demonstrate the circulating antibody (and consequently also the failure to secure a passive transfer with the serum of hypersensitive individuals) does not suffice to justify the claim that this type of hypersensitiveness is independent of antibody. In fact, in many such instances where ordinary methods have failed, it is possible to demonstrate the presence of antibody either by sharpening the methods of detection (such as the use of agglutination of antigen-coated particles instead of attempting to secure precipitation of the antigen directly¹¹) or by securing the antibody from skin extracts or blister fluid.

A particularly important source of confusion in connection with attempts to arrive at the proper correlation between the somewhat aberrant phenomena observed in clinical bacterial allergy and those elicited in hypersensitiveness to relatively simple soluble proteins, is the complexity of physical structure and of the antigenic composition of the bacterial cell. Both when acting as a sensitizing antigen and as a reagent for eliciting the existing state of altered reactivity, bacteria do not act as a single antigen but represent "packages," each containing a variety of independent antigens and haptens. As a result, depending upon the initial portal of entry (and, in final analysis, depending upon the relative rate of disintegration of cells and liberation of independent

antigens), the parenteral introduction of heat-killed suspension of a pure strain of bacteria may result in the production of a variety of antibodies simultaneously or of some at the exclusion of others. Thus, for instance, repeated *intravenous* injection of heat-killed suspension of the smooth variant of pneumococcus of a given type will give rise to type-specific (anticarbohydrate) as well as to species-specific (antiprotein) antibodies, and consequently will bestow upon the skin of the recipient animals the capacity to react to isolated specific carbohydrate derived only from the particular type of pneumococcus, while at the same time it will react to the protein fraction derived from the pneumococcus of any type. The serum of such animals will specifically agglutinate suspensions of this organism and will passively protect mice against infection with homologous type of pneumococcus only.

However, while the specific carbohydrate of these cells is the factor which determines the type specificity of this bacterial antigen, the isolated carbohydrate by itself is devoid of the power to stimulate antibody production unless it is intimately coupled to other constituents, as in the intact bacterial cell. Consequently, intravenous introduction of ground cells, or in any way disintegrated cells, derived from identical cultures fails to stimulate the production of type-specific (anticarbohydrate) antibodies.

Similarly, if the killed suspension of *intact* bacterial cells is introduced repeatedly *into the skin* of normal animals (instead of intravenously as above), such animals also fail to develop type-specific antibodies. Consequently, their serum does not possess the type-specific agglutinins, nor is it capable of conferring passive protection to infection in mice, although the animal (donor of the serum) itself has acquired a degree of resistance to infection not only with homologous, but with any type of pneumococcus. The skin of such animals will fail to react to specific carbohydrate, but will react to the protein fraction derived from pneumococcus of any type.

On the other hand, when bacterial antigens are employed as reagents to elicit the presence of the altered reactivity of the skin, the outcome of such tests frequently may be vitiated, depending upon whether or not the proper antigenic components or the specific haptens are present, and whether they are available in a particular preparation in particulate or in soluble state.

In addition to the above-mentioned constituents, the cell of pneumococcus possesses several other antigenic components. Analogous, though not identical, components are present in all bacterial cells, and not infrequently it happens that some of the constituents of the cells of a given bacterial species are antigenically more or less closely related to the corresponding constituents of the cells of more or less biologically unrelated bacterial species. It is not surprising, therefore, that some of the results of skin tests suggest a relative lack of specificity. Yet with adequate knowledge of the "antigenic spectrum" of the reagents used, these apparently confusing findings may often be adequately interpreted without assuming the existence of a fundamental difference between the respective mechanisms of bacterial and protein allergy. For example, the skin of animals immune to pneumococcus type II will react to extracts of Friedländer type B bacillus, and serum of such animals will protect

normal mice against experimental infection with this organism. This apparently nonspecific phenomenon of bacterial allergy is easily explained by the finding that the specific carbohydrates of these two biologically unrelated organisms are antigenically related. This relationship can be proved by showing that the skin of animals immunized by intravenous introduction of suspensions of Friedländer type B (but not of any other type) bacillus will reciprocally react both in vitro and in vivo with the specific carbohydrate of type II pneumococcus, but not with that of any other type of this organism. Similarly, because *Bacillus proteus* X₁₉ possesses a carbohydrate immunologically related to that present in Rickettsia, the former organism, while utterly unrelated biologically, is routinely employed as a reagent (antigen) in serologic diagnosis of typhus (Weil-Felix reaction).

Furthermore, this complexity of antigenic structure, with all the resulting apparently nonspecific immunologic cross reactivity, is not limited to bacteria, but is found also in the tissues of higher plants and animals. Thus, for instance, the presence of related alcohol-soluble constituents (so-called Forssmann antigen) can be demonstrated by immunologic tests in several unrelated species of bacteria, as well as in corn, beets, turnips, and in certain tissues of chickens, guinea pigs, horses, and in the type A₂ human erythrocytes.

Thus, while this apparent lack of specificity may be confusing, it is not a particular attribute of bacterial allergy, and if not recognized represents a potential source of error in all immunologic reactions with complex antigens. In reality, the apparently aberrant phenomena observed in bacterial allergy (provided all other potential errors* incident to skin-testing are eliminated), far from indicating lack of specificity, on the contrary are the expression of strict specificity which transcends the demarcations of our current classifications.

Still another characteristic which is adduced as a reason for ascribing to bacterial allergy a mechanism distinct from that operating in protein allergy is the lasting effects of desensitization. While specific desensitization is readily accomplished in experimentally sensitized animals, clinical literature is replete with evidence of failure to secure corresponding results in human beings. Moreover, in the case of protein sensitivity in man, in those instances where desensitization is attained, it is less solid (with respect to its intensity) than that exhibited in experimental animals and is of relatively short duration. In contrast to this, while desensitization with bacterial antigens is not more certain of attainment, in cases where it succeeds it is usually found to be more solid and considerably more lasting.

In order to be able to interpret the meaning of these observations, it is necessary first to recall what is the basic mechanism of desensitization in protein anaphylaxis. As the term itself implies, it is generally assumed the failure of desensitized animals to react to injection of antigen is due to inability of tissue cells to anchor the latter because of exhaustion of antibodies

*Such errors as occasioned by differences in age, physiologic condition of the subject, and particularly the physiologic state of the skin (anergy, dermatography); the presence of foci of infection (both specific and unsuspected past or present "undercurrent" infections of subclinical intensity); presence of contaminating unrelated antigens and impairment of specific antigenic reactivity of the reagents which may arise as a result of improper procedures used in their preparation, etc.

at their surface. However, ample evidence indicates that such a concept is incorrect, and consequently the term "desensitization" itself is a misnomer. From the fact that in animals experimentally sensitized to several antigens, repeated injection of one of them reduces the reactivity of these animals to all the other antigens, it is clear that the exhaustion of specific antibodies (even if it should occur) is not the proper explanation. Similar conclusion can be drawn from the observations that no matter how successful, desensitization is never absolute. While "desensitized" animals may fail to react to injection of several times the initially reactive dose of antigen, the injection of still larger amounts will elicit specific response. Furthermore, a comparable degree of refractoriness can be induced in sensitized animals irrespective of the nature of sensitizing antigen (nonspecifically) by parenteral introduction of a great variety of substances both possessing antigenic properties (such as milk, defibrinated autogenous blood, nonspecific bacterial vaccines, etc.) as well as of those devoid of intrinsic immunogenic activity (such as peptone, Bayer 205, lecithin, and in general, substances capable of causing nonspecific "anaphylactoid" reactions). It appears that the degree of effectiveness of this nonspecific refractoriness (desensitization) directly depends upon the degree of systemic or local disturbance evoked by injection. The specific "desensitization" by the introduction of homologous antigen is merely one of the means of producing somewhat analogous disturbance ("colloidoclasia" of Widal), and here again the more intense the reaction resulting from a "desensitizing" injection, the higher is the degree of refractoriness attained. It is for this reason that in animals currently "desensitized" by injection of nearly lethal doses of specific antigen the refractoriness is regularly attained. In human beings we deliberately decrease the dosage (as should be done) in order not to cause any serious disturbance; consequently in order to attain any appreciable degree of refractoriness many such injections are required. The duration of the refractory state so produced directly depends upon the amount of antigen injected to produce it. With bacterial antigens (which are relatively less reactive than soluble proteins) we introduce considerably more reactive material. This is eliminated more slowly and continues to maintain the state of refractoriness through continued combination with antibodies until all antigen is eliminated, when original sensitivity returns just as in the case of hypersensitivity to proteins. It is this residual bacterial antigen which is eliminated very slowly that is responsible for the fact that frequently in chronic infections, or in cases with old incompletely absorbed foci of past infection, the skin tests yield negative findings in spite of the presence of demonstrable antibody.

SUMMARY

The facts brought out in the preceding discussion indicate that the irregularities observed in connection with specific response to intradermal injection of bacterial substrates are not fundamental in nature. They are due largely to the complexity of composition, physical state, and relatively low immunogenic properties of bacterial antigens. The cumulative evidence indicates that the basic mechanism involved in bacterial allergy is identical with that of allergy to simple proteins.

REFERENCES

1. von Pirquet, C.: *Allergie*, Berlin, 1910, Julius Springer, p. 86.
2. Mc Junkin, F. A.: *J. Exper. Med.* 33: 751, 1921.
3. Zinsser, H., and Grinnell, F. B.: *J. Bact.* 14: 301, 1927.
4. Dienes, L., and Schoenheit, E. W.: *Am. Rev. Tuberc.* 20: 92, 1929; *J. Immunol.* 19: 41, 1930.
5. Krauss, R., and Doerr, R.: *Wien. klin. Wchnschr.* 21: 1008, 1908.
6. Krauss, R., and Doerr, R.: *Ztschr. f. Immunitätsforsch. u. exper. Therap.* 3: 130, 1909.
7. Zinsser, H., and Parker, J. T.: *J. Exper. Med.* 26: 75, 1917.
8. Enders, J. F.: *J. Exper. Med.* 50: 777, 1929.
9. Zinsser, H.: *J. Bact.* 14: 301, 1927.
10. Jena, E.: *Ergebn. d. Hyg. Bakt. Immunitätsforsch. u. exper. Therap.* 9: 564, 1928.
11. Freund, J.: *Am. Rev. Tuberc.* 12: 124, 1925; Cannon, P. R., and Marshall, C. E.: *J. Immunol.* 38: 365, 1940.

TREATMENT OF ALLERGIC DISORDERS WITH HISTAMINE AND HISTAMINASE*

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THE essentials of treatment of allergy have not changed much in the last twenty-five years. They depend primarily upon the discovery of the particular foreign substances to which the patient may be hypersensitive. Such a process is usually an involved one and utilizes skin tests, elimination diets, and other forms of environmental control to detect the offending allergen. None of these is consistently dependable, and once the cause of symptoms is discovered, its removal or a defense against it by specific prophylactic injection may by no means be simple. It has, therefore, been the ambition of many to find a way that would circumvent the dependence on a specific immunologic approach. Many therapeutic measures have been introduced for this purpose: injections with colloidal suspensions, bacterial vaccines, glandular substances, x-ray and fever therapy, and a host of others. Among these have been histamine and histaminase.

HISTAMINE

The basis of histamine therapy for allergic disorders is theoretic, and vaguely so. It depends upon the assumption proposed by Dale several years ago that when the particular foreign substance to which man or animal is hypersensitive comes in contact with its specific antibody in the tissue cells, histamine is released. Histamine, in turn, causes smooth muscle spasm and localized edema which are the lesions of anaphylaxis and allergy. Dale's theory has much to support it. Whether it is actually histamine or something akin to it that is released by the tissues is not known. Lewis¹ proposed the term "H-substance" to designate a histamine-like substance.

Although the process by which histamine is elaborated by the tissue cells was unknown, therapy with this substance for allergic disorders was introduced some years ago on the theory that it might act as a type of vaccination, although it had been shown that histamine had no effect in preventing anaphylactic shock in animals. Recently this has been questioned. Nevertheless, histamine was given to patients with bronchial asthma, hay fever, urticaria, and migraine, with indifferent results, and in time this form of therapy was abandoned. In analyzing these early methods of histamine administration, it is noteworthy that injections were given as a rule every few days, which, as will be discussed, may have been the cause of its failure.

During the past three or four years, treatment with histamine has been revived and is receiving considerable support. In contrast to previous experience

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with it, two outstanding facts have emerged: namely, its use is limited largely to chronic urticaria, and initial injections are given daily or even twice a day.

Dzinich² was probably the first to demonstrate that cases of chronic urticaria could be markedly benefited by small daily doses of histamine. The same method was successfully used by several investigators in angioneurotic edema and in patients with so-called "cold allergy,"³⁻⁵ in which edema of the skin occurs on exposure to low temperatures. The initial dose is small, 0.01 mg., which may be built up rapidly to 0.3 mg. After two or three weeks, when improvement occurs, injections are given every other day, then twice a week, and finally are discontinued. Individuals vary in their tolerance to histamine, but when symptoms of overdosage, such as flushing, headache, and palpitation, do occur, they are transitory.

Fiessinger and Gajos⁶ claimed success in the treatment of urticaria by introducing histamine into the skin by iontophoresis. This establishes a depot of the drug, which is slowly absorbed. According to Abrahamsou,⁷ histamine introduced in such a way will remain in situ for several days. Alexander and Elliott⁸ were the first to treat urticaria with histamine given intravenously. The method consists in diluting 0.2 mg. of the base with 10 c.c. of physiologic saline and injecting it slowly. It had been demonstrated by Barsonm and Smirk⁹ that there is a direct ratio between the speed of injection and the amount tolerated. When 6.7 γ were injected in two seconds in a normal subject, facial flushing and acceleration of the heart appeared. No further signs were evident when 333.0 γ were given over a period of 9.3 minutes. In treating urticaria the speed of injection should be such that slight facial flushing and feeling of warmth will occur. The second injection, usually of 0.5 mg., is given the following day, and it was surprising to discover the degree of tolerance that could be established. A third dose of 0.7 mg. may be given the next day. As a rule, urticarial wheals diminish after the first or second dose, and itching becomes less. Even when wheals disappear, intravenous treatment should be supplemented by subcutaneous injections. Otherwise symptoms usually recur within a few weeks.

In the treatment of urticaria with histamine a distinction must be made between cases with discrete spontaneous whealing and those with dermatographism where wheals appear only at pressure areas. The latter cases do not respond to such treatment. The only important supplementary measure is the use of theelin in women who develop urticaria at the time of the menopause. This hormone given alone in such cases is not as effective as when combined with histamine treatment.

Recently, Horton¹⁰ described a type of headache occurring usually in older individuals. Symptoms appear particularly at night while recumbent and are relieved on standing. The pain is very severe, but lasts only a brief time, ten to fifteen minutes. It is always on one side of the head, and is accompanied by vasomotor disturbances as flushing and sweating on the side involved. Histamine given subcutaneously will promptly reproduce the syndrome. When administered in small daily doses as in urticaria, these patients experience marked relief. Histamine therapy has likewise been used for migraine, and although its

effect in this disorder has not been encouraging, sufficient cases wherein the drug was given daily have not been reported to evaluate its merit.

Horton¹¹ also described the successful treatment of Ménière's syndrome by the intravenous injection of histamine. There have been no confirmatory reports.

The literature on histamine treatment is vast, for it includes not only its use in allergic syndromes, but also in various skin manifestations, arthritis, gastrointestinal complaints, neuroses, orthopedic conditions, and many other disorders. Chronic urticaria and angioneurotic edema, types of "physical allergy," headaches, and Ménière's syndrome are the only manifestations associated with allergy in which histamine has been used with any measure of success. Although this form of therapy offers considerable promise, its evaluation should be tentative until supported by further confirmatory series of cases.

The mechanism of histamine therapy is far from understood. A significant fact is that the conditions in which it is successful differ from asthma, hay fever, and atopic eczema in that no extrinsic allergens as pollens, foods, etc., are usually identified. This applies to chronic urticaria, which differs from the acute form which is frequently traced to allergens as shellfish, strawberries, or drugs. Likewise in physical allergy, and in the type of headaches described by Horton, which have some resemblance to migraine, specific allergens are not etiologic factors. Such cases, as well as a small proportion of those of bronchial asthma and vasomotor rhinitis in which no external cause can be identified, have been classified as "intrinsic" allergy, which implies that the cause of symptoms originates within instead of without the body. This is believed to be histamine or H-substance, identical or at least similar to that which is released on contact between allergen and antibody in cases of extrinsic allergy. The simplest demonstration of the ability of cells to release H-substance without the involvement of an immunologic mechanism is seen in the wheal that results from stroking the skin of a dermatographic subject. There are doubtless several conditions which initiate the release of histamine by the tissues.

It appears, then, that histamine therapy is of value in certain manifestations of intrinsic allergy, and to be effective it must be given, at least in the beginning, in daily doses. Why histamine should not alleviate symptoms caused by extrinsic allergens which involve an immunologic mechanism is not understood. The necessity for frequent doses likewise is not clear. This is in keeping, however, with certain related facts. One is the demonstration by Grant and his associates¹² that once H-substance is released by cells of the skin there is a short refractory period, probably less than forty-eight hours, when no further H-substance appears. It is possible that histamine given daily may gradually induce such refractoriness. Again, in the treatment of certain physical allergies as, for instance, urticaria due to cold, frequent exposures to increasingly lowered temperatures by means of baths may quickly ameliorate the whealing. Here, too, treatment must be daily to be effective, which suggests the maintenance of a refractory period. In these cases one may soon arrive at a lowered temperature without production of symptoms which before treatment would have caused violent urticaria. This

demonstration, together with the fact that doses of histamine, particularly when given intravenously, may be increased many fold without symptoms, indicates that a tolerance to histamine introduced from without or to H-substance elaborated from within undoubtedly may be built up. Tolerance to histamine so induced bears a close resemblance to the process of immunization, but since neither true antigens nor antibodies are involved, some other mechanism must be implied. Space will not permit discussion of various possibilities, none of which have as yet been proved. There is much, however, to recommend the participation of histaminase which is produced by certain tissues.

HISTAMINASE

In 1930 Best and McHenry¹³ prepared extracts from intestinal mucous membrane, which when incubated with histamine caused its disappearance. They believed the active agent to be a specific enzyme to which they gave the designation "histaminase." Considerable work has been done on the nature of the reaction, which has been found to be an oxidative process and influenced by various factors, particularly the pH of the menstruum and the amount of available oxygen. In vitro several hours are required for complete inactivation of histamine.

There have been only few observations on the action of histaminase in the intact animal. Where experiments are carefully controlled, it appears that histaminase will within two or three hours completely inactivate all the circulating histamine in the blood of the rabbit, which contains a large quantity in contrast to that of other animals. Moreover, in one hour, histaminase has been found to influence greatly the action of histamine on gastric secretion.¹⁴ Recent work, as yet unpublished, shows that histaminase under given conditions has some inhibiting effect on the production of anaphylactic shock in guinea pigs.

The first clinical report of the use of histaminase appeared in 1935, and for the following three years it had considerable vogue in Germany. It was employed in a variety of disorders as ulcerative colitis, gastric and duodenal ulcer, hepatic cirrhosis, "articular" rheumatism, purpura, acne vulgaris, and several allergic manifestations. In most instances the series of cases was small, and although some therapeutic success was claimed, the evaluation of results in no instance was a critical one.

Histaminase was distributed in this country by its manufacturer for clinical trial in allergic disorders in 1938. Theoretically, there is much to recommend it since it has the property of inactivating histamine completely, both in man and in the intact animal. Inasmuch as histamine or H-substance in man is believed to be the active agent that causes the symptoms of both extrinsic and intrinsic allergy, histaminase, if it could be effectively applied to the tissues involved, should be the ideal substance to check the allergic process. Since its activity is well established and since, also, it is virtually nontoxic when taken in large amounts, the problems of dosage and absorption remain to be worked out.

Clinical reports over the past two or three years of the effect of histaminase in allergic disorders are, with the exception of urticaria, not

sufficiently numerous to permit compilation of adequate statistics of combined experience. A review of this literature reveals the complete absence of any serious study of the determination of dosage, and also the frequent lack of careful judgment in the evaluation of results. With few exceptions, cases of bronchial asthma, hay fever, vasomotor rhinitis, atopic eczema, and gastrointestinal allergy treated with histaminase have, at best, been questionably benefited.

In contrast to these poor results, there is an impressive experience with urticaria. Over 150 cases have been reported, and with the exception of one notable series, the percentage of relief has been surprisingly high, despite a great variation in dosage. In the evaluation of any treatment of urticaria, whether acute or chronic, comment has been made that symptoms often disappear spontaneously, as well as from the psychic effect of the administration of a new drug. However, until the introduction of therapy with histamine and histaminase, treatment of chronic urticaria, in particular, was discouraging.

Analysis of reported cases shows that there was a pronounced effect on many patients in whom urticaria had existed several weeks or longer. There are numerous detailed case histories, as well as compilations from a sufficient number of observers, to be substantial. Piness and Miller¹⁵ recently reported the failure of histaminase in 29 patients with urticaria. An analysis of this series shows that their interpretation seems overly severe in that 6 persons were admittedly relieved promptly, and many others secured partial relief. The authors' conservatism is based on the question whether these patients may not have recovered spontaneously. In contrast to this report, a series of 17 cases of urticaria, all of which were at least of several weeks' duration and had proved refractory to usual therapeutic measures, were studied by Laymon and Cumming.¹⁶ Complete relief was secured with histaminase in 10 patients; and in 2 who had the disease for nine months and several years, respectively, the degree of improvement in the one of 50 per cent and of 90 per cent in the other was recorded. The drug had no value in urticaria factitia or atopic dermatitis.

As with chronic urticaria of unknown etiology, other types of skin edema and whealing have been benefited by histaminase. These include angioneurotic edema, skin manifestations of physical allergy, the urticaria of serum sickness and the localized swelling of insulin allergy at the site of injection. With all these considered as a group, 161 cases by 14 observers have been assembled. Several are single case reports; others are statistical evaluations. Seventy-six, or 47.1 per cent, were recorded as having been completely relieved or markedly improved; 31, or 19.3 per cent, showed definite improvement; and 54, or 33.6 per cent, were not benefited.

From this report it appears that the value of histaminase in the treatment of allergic disorders is distinctly limited. By far the most frequent expressions of allergy are bronchial asthma, hay fever, and vasomotor rhinitis, and in these histaminase has seemed to be of very little value. This result is in rather marked contrast to the reported experience with its use in

urticaria and angioneurotic edema, and it is noteworthy that several observers treated various allergic manifestations with histaminase and singled out the latter as giving good response.

A larger number of cases must be studied under controlled conditions before any claim for the value of histaminase can be established. Of particular necessity is investigation to establish proper dosage. There is much evidence to show that earlier preparations of histaminase were unstable. This fact, as well as very inadequate dosages, makes earlier reports unreliable. Although from the published data there is no established ratio between the degree of relief and the amount of histaminase administered, large doses in all probability will be found necessary.

DISCUSSION

Evaluation of the therapeutic worth of histamine and histaminase in allergic disorders must for the present be tentative. From the reported experience a rather striking similarity is found between the clinical effectiveness of each. Neither appears to be of value in the manifestations of extrinsic allergy as asthma and hay fever, where an immunologic mechanism is involved. Both, on the other hand, are effective in urticaria, angioneurotic edema, and physical allergy, the causes of which are intrinsic in origin. This fact strongly suggests that histamine therapy operates through the production of histaminase. That intrinsic allergy responds to it and the extrinsic form does not, leads to the question whether the H-substance released by the one is qualitatively the same as that elaborated on contact between allergen and antibody. (Serum sickness is extrinsic allergy but of a different mechanism than that of asthma and hay fever.) Indeed, the question whether H-substance is really histamine has frequently been raised, and there are indications that the two are not identical. These are problems as yet unanswered which are engaging the attention of several investigators.

CONCLUSION

In reviewing the effectiveness of histamine and of histaminase therapy in various forms of allergy, the results are disappointing, especially in view of many enthusiastic claims to the contrary. If, however, present statistics will be substantiated, a new form of treatment limited to intrinsic allergy, which is by far the more intractable type, may be at hand. This shows sufficient promise to warrant further investigation.

REFERENCES

1. Lewis, T., and Grant, R. T.: Liberation of a Histamine-like Substance in Injured Skin, *Heart* 11: 209, 1924.
2. Dzinich, A.: Die Histamine Behandlung allergischer Zustände, *Klin. Wchnschr.* 14: 1612, 1935.
3. Bray, G. W.: A Case of Cold Hypersensitivity Treated With Histamine, *J. Allergy* 3: 367, 1931.
4. Saylor, L. L., and Wright, I. S.: Studies on Two Cases of Urticaria From Cold Sensitivity and the Effect of Histamine Treatment, *Am. J. M. Sc.* 192: 388, 1936.
5. Roth, G., and Horton, B. T.: Hypersensitivity to Cold Treatment With Histamine and Histaminase. Report of Case, *Proc. Staff Meet., Mayo Clin.* 12: 129, 1937.
6. Fiessinger, N., and Gajos, A.: *Urticaine et Histamine*, Paris, 1937, Vigot Frères.
7. Abrahamson, H.: Personal communication.

8. Alexander, H. L., and Elliott, R.: To be published.
9. Barsoum, G. S., and Smirk, F. H.: Observations on the Histamine-Yielding Substance in the Plasma of Red Blood Cells of Normal Human Subjects and of Patients With Congestive Heart Failure, *Clin. Sc.* 2: 237, 1936.
10. Horton, B. T., McLean, A. K., and Craig, W. McK.: A New Syndrome of Vascular Headache: Results of Treatment With Histamine, *Proc. Staff Meet., Mayo Clin.* 14: 857, 1939.
11. Horton, B. T.: The Treatment of Ménière's Syndrome by Intravenous Administration of Histamine, *Proc. Staff Meet., Mayo Clin.* 15: 1940.
12. Grant, R. T., Pearson, D., and Comeau, W. S.: Observations on Urticaria Provoked by Exercise and by Warming the Body, *Clin. Sc.* 2: 253, 1935.
13. Best, C. H., and McHenry, C. A.: Inactivation of Histamine, *J. Physiol.* 70: 349, 1933.
14. Suzuki, R.: Experimental Demonstration of the Destruction of Histamine in the Living Body by the Action of Histaminase, *Taiwan Igakkai Zasshi* 36: 68, 1937.
15. Piness, G., and Miller, H.: Histaminase in the Treatment of Allergy, *J. A. M. A.* 114: 1742, 1940.
16. Laymon, C. W., and Cumming, H.: Histaminase in the Treatment of Urticaria and Atopic Dermatitis, *J. Invest. Dermatol.* 2: 301, 1939.

CAPILLARY FACTORS IN PROCESSES OF DISEASE*

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ADVANCES in knowledge of capillary physiology have clarified many obscure circulatory phenomena, both normal and pathologic. A brief summary of endothelial function and capillary reactions is essential to a comprehension of these.

The chief function of the blood, that of supplying oxygen and other substances to the tissue cells, is accomplished by diffusion through the endothelium. A mere increase in the arterial blood flow to an organ is not sufficient. The metabolic needs of the cells are served only when the blood is spread upon the surface of the capillary endothelium. Increased metabolic activity requires an increase in the area of this diffusion membrane. The capillary tubes are so finely drawn that within them 1 c.c. of blood is in contact with from 5,000 to 7,000 sq. cm. of endothelial surface (Krogh¹). If one can imagine 16 drops of fluid evenly spread upon 4 or 5 sq. feet of surface, he may sense the physiologic efficiency with which a minimum volume of blood is spread upon a maximum area of the diffusion membrane. The mechanism regulating both the endothelial area and the flow of capillary blood is marvelous in its delicate adaptability.

Each organ and tissue has a supply of capillaries adequate to its maximal circulatory needs. During functional activity tissues require twenty to forty times more blood than during rest. In resting tissues most of the capillaries are contracted and bloodless. Functioning cells consume oxygen and liberate products of metabolism. Capillary endothelium is delicately susceptible to lack of oxygen. The walls relax and the endothelium becomes more permeable whenever moderate anoxia develops. This relaxation allows an increased flow of arterial blood, supplying oxygen and other nutrients. Fresh arterial blood contains a hormonal substance, perhaps of pituitary origin, which causes the capillaries again to contract. This limits the flow of blood until lack of oxygen and/or the accumulation of metabolites again cause relaxation. Thus the local circulatory cycle is repeated at a rate commensurate with the functional activity of the cells. This self-regulating mechanism, shown in Diagram 1, serves a double purpose: it both distributes blood and adjusts the area of diffusion surface—the endothelial wall—to the local nutritional needs of the cells. In the perfect economy of normal circulation, no excess supply and no unfilled demand exist. The supply is adjusted to the demand with delicate accuracy. The reaction of capillary endothelium to lack of oxygen and to products of metabolism explains both functional and reactive hyperemia.

Capillary reactions are of major importance in the physiologic reaction to tissue injury—inflammation. Ebbecke² showed that any kind of mild injury, mechanical, thermal, electric, chemical or toxic, caused the cells to release cyto-

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plasmic substance. This substance affects the adjacent endothelium, causing an increase in its permeability and leading to dilatation of the capillaries. Lewis and his associates confirmed the observations of Ebbecke. It appears that this response, which is entirely independent of innervation, is physiologic. It initiates the inflammatory reaction which tends toward repair and restoration. The vascular dilatation increases the local blood supply and the permeability allows the escape of plasma into the tissue spaces resulting in local edema. The mechanism by which fibrin is formed locally has been understood for many years, but only recently was it shown³ that cellular substance is chemotropic for leucocytes. These responses explain satisfactorily the mechanism of local acute inflammation.

Abnormal Capillary Reactions.—Three instances of hyperemia, functional, reactive, and inflammatory, occur by virtue of the sensitivity of endothelium to alterations in physiologic conditions. That same quality of sensitivity gives origin to abnormal circulatory phenomena. Heidenhain,⁴ some fifty years ago, noted that commercial peptone would produce a decline in blood pressure accompanied by an increased flow of lymph when given intravenously to dogs. He found that extracts of various marine animals and of normal tissues, as liver, pancreas, mucosa, muscle, and others, produced the same effects. He noted in such experiments that both the volume and the protein content of the lymph were increased and that the plasma volume of the blood was decreased—a change now designated by the term *hemoconcentration*. His observation that the maximum flow of lymph coincided with the period of minimum blood pressure is significant. He stated that concentration of the blood was characteristic of the effects of these “lymphagogues,” and attributed those effects to endothelial secretory activity.

After the lapse of half a century, the full significance of Heidenhain's observations becomes apparent. The theory of endothelial secretory activity was not confirmed (Drinker and Field⁵). The increased flow of lymph, its high protein content, the declining blood pressure, the decreased plasma volume, and the hemoconcentration result from leakage of plasma through endothelium rendered abnormally permeable by the effects of the agents used. Many other substances have been shown to produce the effects described. These include certain poisons of vegetable origin, diphtheria toxin, tuberculin and other bacterial products, foreign proteins and products of protein cleavage, sepsin, histamine, bile and cholic salts, venoms, urethane and other drugs, chemicals, and poisons.

The agents mentioned, and many others not listed, have one property in common: that of producing relaxation and increased permeability of capillary endothelium. If this effect is sufficiently widespread, it causes disturbance of the systemic circulation accompanied by a characteristic clinical syndrome and by equally characteristic morphologic changes in the viscera. The latter consist of dilatation and engorgement of capillaries and venules, occasional petechial hemorrhages resulting from endothelial dissolution, stasis evidenced by masses of closely packed corpuscles in the minute vessels, edema of soft tissues, and acute granular degeneration of parenchymatous organs.

A circulatory deficiency arises if the effects described are extensive. The sequestration of blood in dilated capillaries and venules reduces the *effective* blood volume, while leakage of fluid from the blood into the tissue spaces lowers the *actual* blood volume. This tends to produce a disparity between the volume of blood and the volume capacity of the vascular system, the effects of which resemble somewhat those resulting from extensive hemorrhage. Much confusion has resulted from this resemblance. The two conditions may be differentiated, excepting when they coexist in the same individual, by noting that hemoconcentration is present in the former while the opposite change, hemodilution, develops after hemorrhages.

NORMAL CAPILLARY REACTION

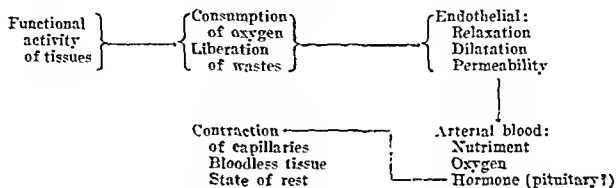


Diagram 1.—The sequence of capillary reactions incident to functional activity in the tissues.

Incipient disparity between the volume of blood and the volume capacity of the vascular bed is compensated for a time by physiologic reactions. Impulses, probably originating in the carotid sinus, activate the sympatho-adrenal system. The discharge of adrenalin into the circulation stimulates the myocardium, mobilizes glucose from the liver, contracts the peripheral arteries, and causes the discharge of reserve blood from the spleen and other reservoirs. So long as this compensation is adequate, there is no ominous decline in the arterial blood pressure, but the latter is maintained at the expense of volume flow. When finally the mechanism of compensation becomes inadequate, the blood pressure declines progressively and the circulatory deficiency is manifested clinically.

Prostration is evident; the patient is profoundly depressed, weak, and restless. The pulse is rapid, feeble, and of small volume. The extremities are cold and the body temperature is low. The face is drawn, ashen or livid in color, anxious in expression, and moist with cold sweat. The eyes are sunken and surrounded by bluish rings, producing the classical "Hippocratic facies." Thirst is incessant, but attempts to relieve it are ineffective because of vomiting. The fluid vomited is often in excess of that swallowed and contains small brown flocculi. Perspiration is profuse, and there may be diarrhea. The respirations are shallow and interspersed with deep sighs. The blood pressure declines progressively. Urination is scanty or suppressed. Consciousness is retained until finally there is loss of sensitivity, of responsiveness to stimuli and reflexes. Unconsciousness or coma precedes death.

The clinical syndrome just described is accompanied by an equally characteristic group of departures from physiologic constants. These are a reduced total and effective blood volume; a reduced minute volume cardiac output and volume flow of arterial blood; hemoconcentration, increased nonprotein nitro-

gen, glucose, and potassium content of the blood; reduced alkaline reserve, chloride and oxygen content, and delayed coagulability of the blood. An increased flow of lymph from the thoracic duct is an early and constant feature.

Hemoconcentration.—This term is used to designate a rapid or sudden increase in the ratio of erythrocytes to plasma per unit volume of blood. It may be detected readily by variations in hematocrit readings, in the erythrocytic count, the hemoglobin content, or in the specific gravity of the whole blood. Variations in the latter occur in a narrower range and hence are less useful as a clinical test than variations in the red blood cell or hemoglobin content of the blood.

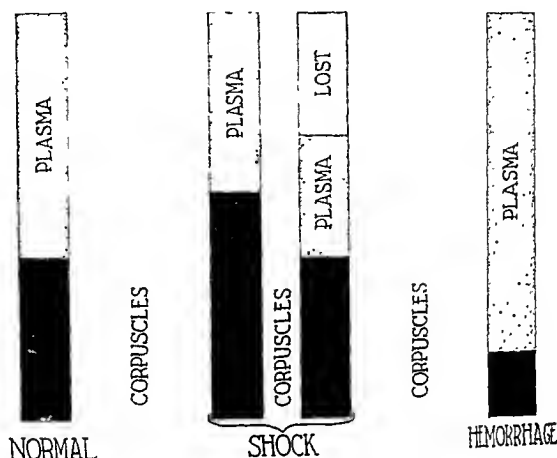


Diagram 2.—Showing a volumetric comparison of normal blood with that present in shock and that resulting from hemorrhage. If normal blood (first column) becomes concentrated 40 per cent, it will have the composition shown in the second column. Seven cubic centimeters of such concentrated blood will contain the same volume of corpuscles (third column) as 10 c.c. of normal blood, 3 c.c. having been lost. In shock with hemoconcentration of 40 per cent, the normal blood has lost 30 per cent of its total volume and 50 per cent of its plasma volume. The fourth column illustrates the hemodilution which occurs when the corpuscles have been reduced by hemorrhages to 40 per cent of the normal.

Hemoconcentration, shown in Diagram 2, is a highly significant feature in the pathologic physiology of shock. Medical literature contains numerous allusions to it but, until recently,⁶ there has appeared no analysis of its relationship to capillary physiology and to circulatory failure. A survey of the recorded observations indicates that the blood regularly becomes concentrated when circulatory failure of capillary origin occurs. This results from loss of plasma volume incident to leakage of fluid into the tissue spaces, due to abnormal permeability of the endothelium. It is integrally related to the increased flow of lymph discussed in a previous section and to the development of edema considered below. It appears that these phenomena occur whenever and wherever the endothelium in an extensive visceral area is damaged. Any type of injury to endothelium renders it abnormally permeable to plasma (Landis⁷).

An extensive increase in the permeability of capillaries deranges the mechanism of water balance and absorption. The mechanism which governs the movement of fluid between the blood and the tissues depends upon the action of several factors, including capillary blood pressure, osmotic pressure, electrolytic concentrations, hormonal substances, and others. But the presence of a normal semipermeable membrane, the endothelium, is absolutely essential to the action

of these forces in preserving a physiologic relationship between intravascular and extravascular fluids. So long as the endothelium is able to perform its part in the maintenance of fluid balance, no hemoconcentration occurs. A serious change in the permeability of the endothelium deranges vitally the mechanism of both water balance and absorption.

This derangement is shown clinically by unmistakable signs. The peripheral tissues are relaxed, inelastic, and have a doughy consistency which suggests dehydration. Vomiting and thirst are incessant, but fluids given by mouth, by rectum, or by hypodermoclysis, are not absorbed into the circulation. Underhill and others⁸ showed that animals about to die of shock resulting from burns of the skin may be injected subcutaneously with lethal doses of strychnine without effect. Normal controls, similarly injected, died from the effects of the strychnine before the burned animals died of shock. Such observations and experiments indicate that the mechanism both of water balance and absorption is seriously deranged when capillary atony has developed extensively or when this type of circulatory deficiency is present. Loss of fluid by vomiting, diarrhea, and perspiration cannot be replaced normally under such conditions. Marked dehydration both of the tissues and of the blood may result from inability of the endothelium to function as a semipermeable membrane.

Abnormal permeability of endothelium is an important cause for edema. It has been shown⁹ that pulmonary edema may be produced experimentally with ease and certainty by injecting any of the numerous agents which have the property of increasing endothelial permeability. Edema resulted regularly when such agents were given in amount sufficient to affect the circulation but not sufficient to cause immediate death. The edema fluid has a high protein content, approximating that of plasma, and is found in the visceral soft tissues and serous cavities. It is rarely found in peripheral tissues. The mechanism of this type of edema is integral with that of shock. It occurs regularly when this condition develops gradually and when death is delayed for several hours.

Pathologic Physiology of Shock.—The type of circulatory disturbance under consideration occurs frequently under diverse clinical conditions, and may be produced experimentally by a wide variety of agents. The mechanism by which the circulatory function is disturbed has been presented diagrammatically in previous articles (see *Note* at end of paper). This diagram is essential to complete the present discussion.

It is seen that a wide variety of clinical and experimental conditions may cause capillary atony and may thereby give rise to circulatory deficiency of capillary origin. I have included under clinical conditions only those with which I have had personal experience. These presented the clinical picture of shock or circulatory collapse, and the post-mortem findings were those regularly found after death by shock. Also under experimental conditions, only those agents have been listed which we have used experimentally. In each instance these agents produced progressive circulatory deficiency accompanied by hemoconcentration and by congestive visceral changes which are characteristic of circulatory failure of capillary origin. Undoubtedly many other conditions, both clinical and experimental, should be added to those listed.

A highly important feature in the mechanism of shock is the fact that lack of oxygen, independent of other conditions, will cause endothelial permeability (Krogh, Ebbecke, Lewis, Landis). This fact introduces a self-perpetuating quality, causing the mechanism to operate as a vicious circle. Hence the circulatory deficiency, unless effectively interrupted in its earlier stages, progresses and leads to irreversible changes.

It is evident from Diagram 3 that the same type of circulatory disturbance may originate primarily from decreased blood volume, decreased volume flow, or from any condition which decreases the supply of oxygen to the tissues below physiologic limits. Under these conditions, lack of oxygen leads to capillary atony and thereby initiates the operation of the same vicious circle.

SUMMARY

It has been shown that capillary endothelium serves indispensable functions under normal physiologic conditions. These include local capillary hyperemia incident to functional activity of tissue cells, inflammatory hyperemia in response to cellular injury, the processes of diffusion and osmosis both in the distribution of useful substances and in the elimination of wastes. Not the least important function of the endothelial membrane has to do with the mechanism of water balance.

The performance of these functions requires that endothelium be delicately sensitive to oxygen tension, to the presence of metabolites and cytoplasmic substances. This very sensitivity renders endothelium a highly vulnerable structure. Its essential quality of semipermeability and its normal tonus are readily affected by diverse agents. These include lack of oxygen, the presence of metabolites in abnormal concentration, products of tissue injury and protein cleavage, bacterial proteins and toxins, venoms, and various drugs, chemicals, and poisons.

The effects of these and similar agents may cause abnormal permeability of a large area of endothelium. This produces a characteristic clinical syndrome of circulatory disturbance, usually called shock or collapse, accompanied by an equally characteristic group of physiologic disorders. The visceral changes seen at necropsy are those indicative of capillary damage. Hemoconcentration is an associated phenomenon which is highly valuable in the recognition of this syndrome in its early stages.

The action of many drugs, chemicals, and poisons is due in part to their effects upon endothelium. It appears that pharmacologic and toxicologic interpretations may need revision to include the effects of many agents upon capillary endothelium.

The self-perpetuating quality of this type of circulatory deficiency makes early recognition essential to successful management. Hemoconcentration is a more useful index than a decline in arterial blood pressure. The former occurs early and is detectable during the incipient stages, while the latter is a sign that the mechanism of compensation has failed.

Efforts to combat shock will be directed logically toward the removal of the cause, toward the restoration of the lost blood volume and capillary tonus, and toward relieving anoxia.

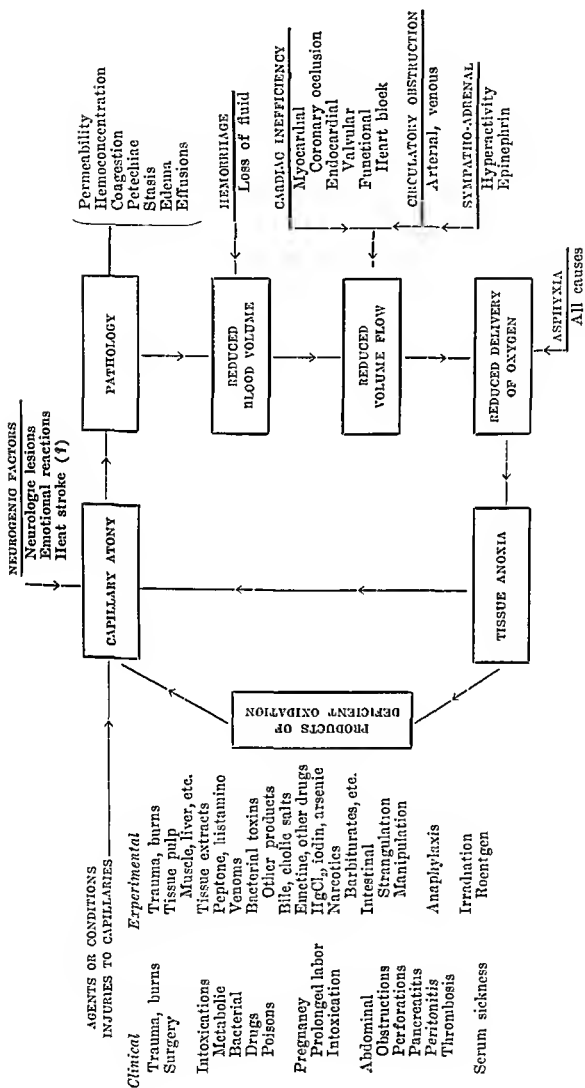


Diagram 3.—The relationship of various factors in the pathologic physiology of shock. The reciprocal effects of capillary atony and tissue anoxia give this mechanism the self-perpetuating quality of a vicious circle.

Unless the condition that caused the circulatory deficiency is relieved, any efforts to restore circulatory efficiency will be useless. Transfusions of plasma or serum are more suitable than whole blood, because they relieve the hemoconcentration by supplying the same type of fluid which was lost. There is physiologic evidence that *one* function of the adrenal cortical hormone is the maintenance of endothelial tonus. Hence the use of cortical extract during the incipient stages of shock is logical. Recent reports of its use in experimental and in clinical shock indicate beneficial effects. The inhalation of oxygen may retard the progress of the circulatory deficiency, since anoxia supplies the self-perpetuating quality in the mechanism of shock.

NOTE.—Much of the subject matter here presented is condensed from previous publications. Readers desiring further details and bibliography are referred to Shock and Related Capillary Phenomena, New York, 1938, Oxford University Press; Occurrence and Clinical Significance of Hemoconcentration, *Ann. Int. Med.* 13: 451-475, 1939; Circulatory Failure of Capillary Origin, *J. A. M. A.* 114: 1312-1318, 1940.

REFERENCES

1. Krogh, A.: *Anatomy and Physiology of the Capillaries*, ed. 2, New Haven, 1929, Yale University Press.
2. Ebbecke, U.: Die lokale vasomotorische Reaktion der Haut und der inneren Organen, *Arch. f. d. ges. Physiol.* 169: 1, 1917.
Ueber Gewebsreizung und Gefäßreaktion, *Ibid.* 199: 197, 1923.
Capillärerweiterung. Urticaria und Schock, *Klin. Wchnschr.* 2: 1725, 1923.
3. Silverman, D.: A Chemotropic Substance Derived From Normal Tissues, *Arch. Path.* 25: 40, 1938.
4. Heidenhain, R.: Versuche und Fragen zur Lehre der Lymphbildung, *Arch. f. d. ges. Physiol.* 49: 209, 1891.
5. Drinker, Cecil K., and Field, M. E.: *Lymphatics, Lymph and Tissue Fluid*, Baltimore, 1933, Williams & Wilkins.
6. Moon, V. H.: The Occurrence and Clinical Significance of Hemoconcentration, *Ann. Int. Med.* 13: 451, 1939.
7. Landis, E. M.: Capillary Pressure and Capillary Permeability, *Physiol. Rev.* 14: 404, 1934.
8. Underhill, F. P., Carrington, G. L., Kapsinow, Robert, and Pack, G. T.: Blood Concentration Changes in Extensive Superficial Burns and Their Significance for Systemic Treatment, *Arch. Int. Med.* 32: 31, 1923.
9. Moon, Virgil H., and Morgan, D. R.: Experimental Pulmonary Edema, *Arch. Path.* 24: 642, 1936.
10. Lewis, T.: *Blood Vessels of the Human Skin and Their Responses*, London, 1927, Shaw & Sons.

POSSIBLE SIGNIFICANCE OF THE INHIBITORY EFFECT OF FEVER ON ANAPHYLACTIC PHENOMENA*

*"Fever is a mighty engine which Nature brings
into the world for the conquest of her enemies."*

—Sydenham (1624-1689)

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WHEN Dr. Warren T. Vaughan, Editor of this JOURNAL, invited us to prepare a paper for the special issue commemorating the twenty-fifth anniversary of its founding by his father, our beloved teacher, Dr. Victor Clarence Vaughan, it occurred to us that we should select a subject in which our interest was first aroused by his researches and teachings. This subject is anaphylaxis. Our interest in this field was revived and augmented by certain observations made in the course of studies on artificial fever therapy during the past nine years.

We have, in general, concentrated our fever studies upon those diseases in which investigation had already shown fever—intercurrent, or by inoculation, or by physical means—to have a favorable effect. In our experience these are syphilis, gonococcic infections, chorea, and other manifestations of the rheumatic state, brucellosis, and certain forms of infectious arthritis. Despite the wide variety and disparity of known or alleged etiologic agents, and the absence of any uniformity in the manifestations of these diseases, it is now generally admitted that fever has a beneficial influence on all of them. Many theories have been advanced in an attempt to explain these favorable effects of high body temperatures. Most observers admit, however, that the therapeutic effect of fever rests on an entirely empirical basis.

A generation ago most physicians regarded fever as an alarming manifestation of disease. Antipyretic drugs were widely employed. The past ten years have witnessed a revolution in the concept of the role of fever. It is now known that it is one of Nature's most important weapons of defense. The failure to discover a rational explanation of the defensive action of fever is unfortunate. The acceptance of its beneficial effects has been inhibited by their mystery. Knowledge of the mechanism by which fever acts would not only hasten its wider acceptance as a therapeutic agency, but would also promote further researches toward possible wider therapeutic applications.

There is no doubt that the dangers and the uncertainty of control of fever in the past have maintained the empiricism from which the use of fever has not yet been freed. Medical literature is replete with citations of the beneficial influence of intercurrent infections accompanied by sustained high fever on such

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diseases as chorea, asthma, paresis, and gonorrhea. But it was not until the development of safe and controllable physical methods of producing fever artificially that a scientific tool became available to study the effects of high body temperatures per se. Now the first step toward penetrating beneath empiricism could be taken since the toxic effects of invading organisms present in intercurrent infections were absent.

I. CLINICAL OBSERVATIONS ON THE EFFECT OF HIGH BODY TEMPERATURES ON CERTAIN SENSITIZATION PHENOMENA

(a) *Arsenic Sensitization in Therapy of Syphilis.*—During the first three years (1931-1934) of our experimental studies on the treatment of syphilis by combined artificial fever and chemotherapy,¹⁻⁵ we became gradually aware of the fact that there was an absence of the anticipated proportion of sensitization reactions to arsenical compounds. In time we were forced to the conclusion that fever in some way suppresses such arsenic sensitiveness, and we began to experiment with injections of arsenical compounds during artificial fever into syphilitic patients known to be extremely sensitive to arsenic.

Such a trial appeared to be justified in a young man with primary syphilis who developed severe generalized exfoliative dermatitis following the second injection of neoarsphenamine, ten days after the appearance of a penile chancre.⁴ Because of this patient's marked idiosyncrasy to arsenic, no chemotherapy was combined with the first four fever treatments. The exfoliative lesions disappeared after the third fever treatment. Before the fifth fever treatment was given, the patient was tested with 1/1,000 Gm. of neoarsphenamine. Two days later exfoliation again developed. At the beginning of the fifth fever treatment, when the rectal temperature reached 105° F. (40.6° C.), a similar quantity (1/1,000 Gm.) of neoarsphenamine was injected intravenously. This injection was not followed by exfoliative dermatitis.

During succeeding fever sessions 0.1 Gm. injections of bismarsen were given when the rectal temperature reached 105° F. (40.6° C.). Dermatitis did not develop. Because of this patient's extreme sensitiveness to arsenic, when it was given without the protection of fever, the usual follow-up course of chemotherapy alone was omitted.

Despite the small total quantity of arsenic given in combination with artificial fever, this patient's blood and spinal fluid reactions, strongly positive before fever therapy was instituted, were reversed to negative, and have remained so for four years. Since 1935 it has become our routine practice to treat all patients, referred to us because their arsenic sensitiveness rendered them untreatable by orthodox chemotherapy, with full adult doses of arsenicals under the protection of artificial fever.

Encouraged by these observations on the protective function of fever in suppressing arsenical reactions, we were led to initiate experimental studies designed to shorten greatly the treatment time of early syphilis. Preliminary observations appear to justify the hope that by combining large doses of arsenic and bismuth with one or two long fever sessions, it may be possible to reduce the minimum treatment time from eighteen months to one or two days.

(b) *Artificial Fever Therapy of Chorea and Other Manifestations of the Rheumatic State.*—From 1930 to 1935, in a field apparently far removed from

that of arsenic sensitivity, many workers were making empirical observations on the effect of fever upon a human disease condition of which the mechanism is allergic, or, more specifically, anaphylactic. This is the rheumatic state.

It is generally agreed that the outlook for patients suffering from Sydenham's chorea has improved remarkably during the past ten years. The experience of many workers with various forms of fever therapy, first introduced by Sutton and Dodge⁶ in 1930, indicates that it may be possible not only to abolish the distressing choreatic symptoms, but also to influence favorably other more specifically rheumatic manifestations. We do not here maintain that chorea is invariably the result of rheumatic activity, yet it is generally so regarded when it is accompanied by migratory polyarthritis, and especially by carditis.

All these rheumatic exacerbations are favorably influenced to a greater or less extent by fever. There is no longer doubt that the common denominator of the variety of antirheumatic agencies—such as triple-typhoid vaccine, sterile milk injections, inoculation with relapsing fever—is the production of sustained high body temperatures. Today the various fever-producing inoculations have largely given way to the safer and more accurately controllable artificial fever by physical means.

The notable clinical improvement in rheumatic carditis observed by a number of workers⁷⁻¹¹ is confirmed by marked trends toward normal of heart function, as revealed by accompanying electrocardiographic, roentgenographic, and cardiovascular functional studies. Furthermore, it has been reported by Dunn and Simmons⁹ that the high erythrocyte sedimentation rates, so characteristic of rheumatic activity and of subclinical rheumatic smoldering, are reduced toward normal by artificial fever treatment. These observers believe, from the clinical improvement in carditis and the return toward normal of the laboratory findings (white blood cell counts and erythrocyte sedimentation rates), that most rheumatic patients terminate their active disease more rapidly when treated with artificial fever than when treated with bed rest and large doses of salicylates. They feel that by shortening the period of the rheumatic cardiac exacerbations they are able to minimize the accumulating cardiac damage to rheumatic sufferers.

These findings, confirmed by our own experience,¹² suggest a link between the effect of high body temperatures upon arsenic sensitivity on the one hand, and upon the rheumatic state on the other. For the observations of many workers, beginning with Weintraud¹³ and culminating with the brilliant researches of Coburn during the past ten years, indicate that rheumatism is essentially an anaphylactic condition. During this ten-year period the work of Coburn has brought an enormous number of facts to the support of his original view¹⁴ that certain "effective" strains of the hemolytic streptococcus are causal agents of rheumatic fever, but operate in a different manner, in a pathogenetic sense, from any microbial agents so far known.

As happily and succinctly put by Collis,¹⁵ a great deal of evidence has now accumulated to support the theory that rheumatic disease is caused by an antigen-antibody reaction occurring in certain people born with the rheumatic diathesis or in those who acquire it.

If the various rheumatic insults are, then, expressions of what may be termed "micro-anaphylactic shocks" in mesodermal tissues, is it not possible that the hitherto empirical observations of the favorable effect of high body temperatures upon the various rheumatic exacerbations (chorca, migrating polyarthritis, carditis) may have a rational explanation?

In sum, is it not conceivable that body temperatures, raised to a certain level and maintained there, may not in some way disrupt or nullify the noxious antigen-antibody reaction responsible for rheumatic damage?

(c) *Effect of Artificial Fever on Intrinsic Intractable Asthma and Other Allergic States.*—The age-old observation that the advent of an intercurrent febrile disease, such as scarlet fever, erysipelas, or pneumonia, often resulted in temporary remission or improvement of chronic intractable asthma led Feinberg and his associates¹⁶ to treat with artificial fever a group of 42 patients with intrinsic, intractable asthma. All had failed to respond to accepted modern therapy. Of 35 patients, the result of whose treatment could be ascertained, 51 per cent had complete remission, varying from several days to nine and one-half months, while an additional 39 per cent were appreciably improved. Similarly, favorable responses have been reported by Sheldon,¹⁷ Metz,¹¹ Bennett,¹⁰ Brodribb,¹⁸ Cook,¹⁹ and ourselves.

There is also suggestive evidence that artificial fever therapy exerts a favorable influence upon certain patients suffering from seasonal hay fever, chronic urticaria, and chronic eczema.^{16, 20}

II. INHIBITION OF CLASSICAL ANAPHYLACTIC SHOCK IN GUINEA PIGS BY HIGH BODY TEMPERATURES

By 1936 it had become clear to us that it might be possible to integrate these widely disparate and scattered observations of the inhibitory effect of fever upon the phenomena of arsenic sensitivity, rheumatism, allergic asthma, hay fever, and eczema. In short, if these various disease phenomena are in essence anaphylactic, and if they are more or less controlled and suppressed by fever, then the induction of suitably high temperatures in sensitized experimental animals should inhibit anaphylactic shock phenomena that would otherwise certainly result when the specific antigen was introduced in an effective concentration into their veins.

We laid plans for this crucial integrating experiment in 1936, but pressure of other work delayed putting these plans into experimental action. Meanwhile, Goldman,²¹ of the University of Cincinnati (one of the 29 institutions collaborating with the Kettering Institute for Medical Research in our fever therapy research project), published observations on the inhibitory effect of high body temperatures in still another field remote from those already mentioned. He demonstrated an inhibitory effect of very high fever (108° to 112° F.; 42.2° to 44.4° C.) upon experimental sensitization of guinea pigs to turpentine.

In the discussion of Goldman's paper (read before the Second Annual Meeting of the Society for Investigative Dermatology at St. Louis, Mo., May 16, 1939) I. Arthur Mirsky, together with P. Wassermann, who were associated with Goldman in his fever-turpentine studies, reported their attempt to find some definite mechanism in explanation of his observations. These workers found

that a series of guinea pigs, sensitized to horse serum, and subsequently febrile to temperatures of 109°-110° F. (42.8° to 43° C.), failed to respond with anaphylactic shock when injected intracardially with doses of horse serum that called out typical anaphylactic shock in nonfebrile, sensitized control guinea pigs. These experiments appeared to vindicate our hypothesis that high body temperatures do indeed suppress the classical anaphylactic shock of guinea pigs.

During the past year, in collaboration with R. Gottschall and C. C. Young at Michigan State Health Department Laboratories, Lansing, we have been able to launch a detailed experimental analysis of our hypothesis. In the first place, we were concerned with the extremely high temperatures employed by Goldman, Mirsky, and Wassermann. Such temperatures are not compatible with life in man. Furthermore, clinical observations on suppression of anaphylactic phenomena by fever were based upon temperature ranges which were administered with safety and relative comfort to human beings.

Only by using such temperatures in our experiments on the possible inhibiting action of fever upon classical anaphylaxis, would this crucial animal experiment have significance in integrating the hitherto empirical observations on the favorable influence of fever upon several unrelated human disease conditions.

Preliminary observations, accumulated during our experimental studies now being conducted at Lansing, Mich., leave little doubt that suppression of anaphylactic shock in guinea pigs does occur in the temperature ranges ordinarily employed in fever therapy of human beings.

In these experiments diligent efforts are being made to eliminate error by the use of guinea pigs of pure genetic strain, and by employment of instruments of precision in recording temperatures. The details of these studies will be made the subject of a later communication.

SUMMARY AND CONCLUSIONS

1. The lack of any rational explanation for the favorable influence of fever upon several unrelated disease states stimulated us to search for some basic underlying mechanism for the therapeutic action of fever.
2. The common denominator in the favorable effect of fever in such clinically different conditions as sensitiveness to arsenic, chorea, and other manifestations of the rheumatic state, and asthma and other allergic phenomena, appears to be the inhibition of anaphylaxis.
3. It is suggested that the phenomenon of inhibition of classical anaphylaxis in the guinea pig by body temperatures at levels safely employed in fever therapy of human beings may provide a crucial experimental explanation of these hitherto empirical therapeutic effects.

REFERENCES

1. Simpson, W. M., Kislig, F. K., and Sittler, E. C.: Ultra-High Frequency Pyretotherapy of Neurosyphilis: A Preliminary Report, *Ann. Int. Med.* 7: 64, 1933.
2. Simpson, W. M.: Artificial Fever Therapy of Syphilis, *J. A. M. A.* 105: 2132, 1935.
3. Simpson, W. M., and Kendell, H. W.: Artificial Fever Therapy, *Colorado Med.* 34: 782, 1937.
4. Simpson, W. M., and Kendell, H. W.: Experimental Treatment of Early Syphilis With Artificial Fever Combined With Chemotherapy, *Am. J. Syph., Gonorr. & Ven. Dis.* 21: 526, 1937.

5. Culler, A. M., and Simpson, W. M.: Artificial Fever Therapy in Cases of Ocular Syphilis, *Arch. Ophth.* 15: 624, 1936.
6. Sutton, L. P.: The Treatment of Chorea by the Induction of Fever, *J. A. M. A.* 97: 299, 1931.
Sutton, L. P., and Dodge, K. G.: Treatment of Chorea by Induced Fever, *J. Pediat.* 3: 813, 1933; Fever Therapy in Chorea and in Rheumatic Carditis, *Proc. First International Conference on Fever Therapy*, New York, 1938, Paul B. Hoeber, Inc., p. 73; Fever Therapy in Chorea and in Rheumatic Carditis With and Without Chorea, *J. LAB. & CLIN. MED.* 11: 619, 1936.
7. Barnacle, C. H., Ewalt, J. R., and Ebaugh, F. G.: Artificial Fever Treatment of Chorea, *J. A. M. A.* 106: 2046, 1936; Artificial Fever Treatment of Chorea, *Proc. First International Conference on Fever Therapy*, New York, 1938, Paul B. Hoeber, Inc., p. 77; Artificial Fever Treatment of Chorea: A Two Year Study, *J. A. M. A.* 109: 111, 1937.
8. Neymann, C. A., Blatt, M. L., and Osborne, S. L.: The Treatment of Chorea Minor by Means of Electropyrrexia, *J. A. M. A.* 107: 938, 1936.
Osborne, S. L., Blatt, M., and Weymann, C. A.: Electropyrrexia in Rheumatic Carditis, Chorea, and Certain Other Childhood Diseases, *Proc. First International Conference on Fever Therapy*, New York, 1938, Paul B. Hoeber, Inc., p. 68.
9. Simmons, E. E.: Value of Fever Therapy in the Treatment of Arthritides, *Am. J. M. Sc.* 194: 170, 1937.
Simmons, E. E., and Dunn, F. L.: Fever Therapy in the Treatment of Acute Rheumatic Fever, *Proc. First International Conference on Fever Therapy*, New York, 1938, Paul B. Hoeber, Inc., p. 66.
10. Bennett, A. E.: Artificial Fever Therapy. *Practitioner's Library Supplement*, Appleton-Century Co., New York 48: 657, 1937.
11. Metz, M. H.: Some Unusual Cases Treated With Fever Therapy, *Rep. Fifth Ann. Fever Conference*, 1935, p. 101; Results Obtained by Use of Fever Therapy in Three Types of Cases Usually Refractory to Other Types of Treatment, *J. A. M. A.* 106: 1658, 1936.
12. Kendell, H. W., and Simpson, W. M.: Artificial Fever Therapy of Sydenham's Chorea, *Ohio State M. J.* 33: 1097, 1937.
13. Weintraud: Cited by Levinthal, W.: Aetiological Problem of Rheumatism, *Ann. Rheumat. Dis.* 1: 67, 1939.
14. Colburn, A. F.: The Factor of Infection in the Rheumatic State, Baltimore, 1931, Williams & Wilkins Co.
15. Collis, W. R. F.: Bacteriology of Rheumatic Fever, *Lancet* 2: 817, 1939.
16. Feinberg, S. M., Osborne, S. L., and Afremol, M. L.: A New Method of Non-Specific Treatment of Allergic Disease: Preliminary Report, *J. Allergy* 2: 291, 1931; Fever by Diathermy in the Treatment of Allergic Disease, *J. Allergy* 2: 415, 1931.
Feinberg, S. M., Osborne, S. L., and Steinberg, M. J.: Sustained Artificial Fever in the Treatment of Intractable Asthma, *J. A. M. A.* 99: 901, 1932.
17. Sheldon, J. R.: Results of Fever Therapy in Intrinsic Intractable Asthma. A Preliminary Report, *Proc. Fifth Fever Conference*, 1935, p. 83.
18. Brodribb, H. B.: Artificial Fever Produced by Physical Agents, *St. Barth. Hosp. Rep.* 70: 79, 1937.
19. Cook, M. M.: Report of Three and One-half Years' Experience With Artificial Fever Therapy. *Staff Meeting Bull., Hospitals of the University of Minnesota* 10: 418, 1939.
20. Ferderber, M. B.: Personal communication to the authors.
21. Goldman, L.: The Effect of Fever Artificially Induced on the Skin Sensitivity of Guinea-pigs to Turpentine, *J. Invest. Dermat.* 2: 281, 1939.

DEVELOPMENT OF NEW ORGANIC CHEMICALS FOR THERAPEUTIC USE

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A COMPARISON of the various substances used as medicaments about 1500 B.C., as disclosed in the Eber's papyrus, with those mentioned in three editions of the London Pharmacopoeia, which appeared during the years 1618 and 1766, reveals the fact that practically no progress was made in the field of therapeutic agents for over three thousand years. In contrast to this era of stagnation, the discoveries made during the last fifty years are truly astonishing.

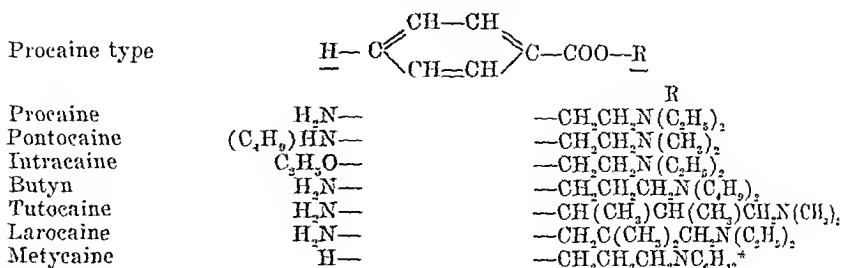
It is difficult to conceive of any development which has been of greater benefit to the human race than that which has taken place in synthetic drugs, and it should not be forgotten that this progress could never have been made without the growth and vast accumulation of data in sciences such as chemistry, physics, bacteriology, and biology.

It seems impossible to devise a rigid, sharply defined classification of synthetic, organic therapeutic agents. Probably the most satisfactory arrangement is one in which the various medicaments are grouped in accordance with their medicinal use. Each major group may then be divided into subgroups on the basis of some characteristic feature in their chemical structure. It will be noticed that the greatest variations in structure are to be found in substances which possess similar therapeutic properties and that compounds of analogous structure sometimes exhibit very different types of physiologic activity.

General Anesthetics.—Ether was used first in surgery in 1842 and chloroform was introduced five years later. Seventy-six years passed before another anesthetic of practical value was discovered, although during this time constant improvement was made in the method of administration of the two drugs. In 1923 ethylene was adopted, followed by avertin (β, β, β -trichloroethyl alcohol), cyclopropane, and vinethene (divinyl oxide). These newer anesthetics possess certain undeniable advantages, but the fact that some of them, especially ethylene and cyclopropane, form very explosive mixtures with oxygen limits their use.

Local Anesthetics.—It was discovered, soon after its introduction in 1905, that procaine (novocain) can be used as a cocaine substitute most effectively and advantageously for infiltration anesthesia. However, for surface anesthesia it proved to be greatly inferior to the plant drug. To some extent this disadvantage has been overcome by the synthesis of products which, although of the procaine type, differ somewhat from the latter substance in structure; some of these are much better surface anesthetics than procaine.

So far, all attempts to obtain a local anesthetic which will produce constriction of the capillaries, and thus make the concomitant use of a vasoconstrictor unnecessary, have been unsuccessful.



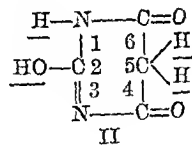
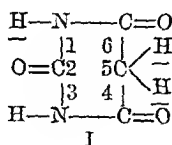
* NC_6H_{12} is the 2-methyl-1-piperidyl radical.

Other local anesthetics which should be mentioned are psicaine, psicaine-new, nupercaine, diothane, apothesine, and phenacaine.

Although their low water-insolubility makes them unsuitable for injection, compounds such as anesthesin, butesin, and orthoform are of considerable value for topical application.

Hypnotics.—Most of the hypnotics which are widely used at the present time are amides, a type which was introduced many years ago. Among these we find the substituted acetamides, neuronal, neodorm, and novonal; the acylureas, carbromal, bromural, and sedormid, and especially cyclic amides of the barbituric acid series. Very few new compounds of the acetamide or urea groups have appeared on the market, but year after year new representatives of the barbituric acid series have been introduced until at present more than twenty-four of these products are available.

DERIVATIVES OF BARBITURIC ACID



Formula I

	1	2	5	(5, 5)
Evipala	CH_3-	$-\text{CH}_3$	$\begin{array}{c} \text{CH}-\text{CH}_2 \\ \text{C} \quad \text{CH}_2 \\ \text{CH}_2-\text{CH}_2 \\ \text{CH}_2-\text{CH}_2 \end{array}$	methyl, cyclohexenyl
Barbital ^b	H	$-\text{CH}_2\text{CH}_3$	$-\text{CH}_2\text{CH}_3$	ethyl, ethyl
Neonal	H	$-\text{CH}_2\text{CH}_3$	$-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$	ethyl, butyl
Amytal	H	$-\text{CH}_2\text{CH}_3$	$-\text{CH}_2\text{CH}_2\text{CH}(\text{CH}_3)_2$	ethyl, isoamyl
Phanodorn		$-\text{CH}_2\text{CH}_3$	$\begin{array}{c} \text{CH}-\text{CH}_2 \\ \text{C} \quad \text{CH}_2 \\ \text{CH}_2-\text{CH}_2 \\ \text{CH}_2-\text{CH}_2 \end{array}$	ethyl, cyclohexenyl
Phenobarbital ^c	H	$-\text{CH}_2\text{CH}_3$	$-\text{C}_6\text{H}_5$	ethyl, phenyl
Mebaral ^d	CH_3-	$-\text{CH}_2\text{CH}_3$	$-\text{C}_6\text{H}_5$	ethyl, phenyl
Alurate	H	$-\text{CH}(\text{CH}_3)_2$	$-\text{CH}_2\text{CH}=\text{CH}_2$	isopropyl, allyl
Nostal	H	$-\text{CH}(\text{CH}_3)_2$	$-\text{CH}_2\text{CBr}=\text{CH}_2$	isopropyl, β -bromoallyl
Dial	H	$-\text{CH}_2\text{CH}=\text{CH}_2$	$-\text{CH}_2\text{CH}=\text{CH}_2$	allyl, allyl
Sandoptal	H	$-\text{CH}_2\text{CH}(\text{CH}_3)_2$	$-\text{CH}_2\text{CH}=\text{CH}_2$	isobutyl, allyl
Pernoston	H	$-\text{CH}(\text{CH}_3)_2$	$-\text{CH}_2\text{CBr}=\text{CH}_2$	sec.-butyl, β -bromoallyl

Formula II

Evipal soluble	CH ₃ -	NaO—	—CH ₃	$ \begin{array}{c} \text{CH} - \text{CH}_2 \\ \diagup \quad \diagdown \\ -\text{C} \quad \text{CH}_2 \\ \diagdown \quad \diagup \\ \text{CH}_2 - \text{CH}_2 \end{array} $	methyl, cyclohexenyl
Medinal*	H	NaO—	—CH ₂ CH ₂	—CH ₂ CH ₂	ethyl, ethyl
Ipral sodium	H	NaO—	—CH ₂ CH ₂	—CH(CH ₃) ₂	ethyl, isopropyl
Ipral calcium	H	—OCaO—	—CH ₂ CH ₂	—CH(CH ₃) ₂	ethyl, isopropyl
Nembutal	H	NaO—	—CH ₂ CH ₂	—CH(CH ₃)CH ₂ · CH ₂ CH ₂	ethyl, 1-methylbutyl
Pentothal sodium	H	NaS—	—CH ₂ CH ₂	—CH(CH ₃)CH ₂ · CH ₂ CH ₂	ethyl, 1-methylbutyl
Sodium amytal	H	NaO—	—CH ₂ CH ₂	—CH ₂ CH ₂ CH· (CH ₃) ₂	ethyl, isoamyl
Ortal sodium	H	NaO—	—CH ₂ CH ₂	—CH ₂ CH ₂ CH ₂ · CH ₂ CH ₂ CH ₂	ethyl, hexyl
Luminal sodium	H	NaO—	—CH ₂ CH ₂	—C ₆ H ₅	ethyl, phenyl
Dormaphen	H	—OMgO—	—CH ₂ CH ₂	—C ₆ H ₅	ethyl, phenyl
Sodium alurate	H	NaO—	—CH(CH ₃) ₂	—CH ₂ CH=CH ₂	isopropyl, allyl
Cyclopal sodium	H	NaO—	—CH ₂ CH=CH ₂	$ \begin{array}{c} \text{CH} = \text{CH} \\ \diagdown \quad \diagup \\ -\text{CH} \quad \text{CH}_2 \\ \quad \quad \\ \quad \quad \text{CH}_2 \end{array} $	allyl, cyclopentenyl
Seconal	H	NaO—	—CH(CH ₃)CH ₂ · CH ₂ CH ₂	—CH ₂ CH=CH ₂	1-methylbutyl, allyl
Sigmoidal	H	NaO—	—CH(CH ₃)CH ₂ · CH ₂ CH ₂	—CH ₂ CB ₂ =CH ₂	1-methylbutyl, β-bromo-allyl

(a) Another name is cyclural; (b) veronal; (c) luminal; (d) prominal; (e) veronal sodium, soluble barbitol.

Because of the variety of hypnotics which can now be obtained, the physician has available drugs which are so mild in their effect that they may be classed more properly as sedatives; hypnotics which act very rapidly and others with delayed action; drugs which act over a short or a long period of time; and, finally, soluble products which, after intravenous injection, produce relaxation and narcosis sufficient for major surgery.

Antispasmodics.—During the last ten years more than five hundred compounds have been synthesized and examined pharmacologically in the hope that a product might be found which would be more effective than the naturally occurring antispasmodics atropine and papaverine. The following compounds have appeared on the market: novatropine, syntropan, trasentin, dolantin, eupaverin, perparin, new-paverin, octin, sestron and cyverine hydrochloride; those introduced most recently are cyverine hydrochloride and dolantin. The first four compounds resemble atropine to the extent that they are basic esters; the others, like papaverine, are amines. In general, the activity of the synthetic antispasmodics has been most pronounced in cases of spasms in the gastrointestinal tract. The antispasmodic is still to be discovered which will relieve spasms of both musculotropic and neurotropic character in all types of smooth musculature.

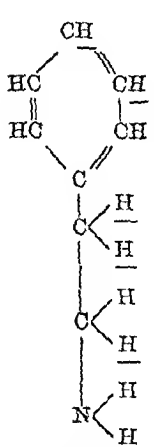
Compounds Which Stimulate Cell Proliferation.—The fact that maggots (*Lucilia sericata*) can be employed effectively to remove necrotic tissue and prevent bacterial putrefaction is well known. In earlier times the comfrey root was used to promote healing of wounds. Some years ago it was found

that allantoin, which stimulates the growth of epithelial tissue, is present in maggot excretions as well as in the comfrey plant.

A number of compounds which differ greatly from one another in chemical structure have been found to act similarly to allantoin, for example, thioercol, thioglycerol, urea, and certain azo dyes. The manner in which these compounds promote tissue growth is not known definitely but an important factor in their beneficial effect seems to be the solvent action on necrotic debris which some of them exhibit.

Pressor Agents.—The prevailing tendency to prepare and substitute synthetic drugs for those obtained from natural sources is illustrated again in the case of pressor agents which are listed below. All of the products may be regarded as derivatives of β -phenylethylamine.

DERIVATIVES OF β -PHENYLETHYLAMINE

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
	H	H	H	NH ₂	OH	OH	OH	H	OH	OH	OH	OH
	H	H	H	H	H	H	H	OH	OH	OH	OH	OH
	H	H	H	H	H	H	H	H	H	H	H	O
	H	OH	OH	OH	H	H	OH	OH	H	OH	OH	
	H	H	H	H	H	H	H	H	H	H	H	H
	CH ₃	CH ₃	CH ₃	CH ₃	H	CH ₃	H	H	H	H	CH ₃	H
	H	H	H	H	H	H	H	H	H	H	H	H
	H	H	CH ₃	CH ₃	H	H	CH ₃	CH ₃	CH ₃	CH ₃	H	CH ₃

β -Phenylethyl-amine

I Benzedrine

II Propadrine

III Ephedrine

IV Ephetonal

V Tyramine

VI Paredrine

VII Synephrin

VIII Neosynephrin

IX Epinine

X Epinephrine (Adrenaline)*

XI Cobefrin (Corbasil)

XII Kephirin

*These naturally occurring drugs can all be manufactured by synthetic processes, and tyramine is obtained entirely in this manner. Whether market supplies of epinephrine and ephedrine are synthesized or extracted from natural products depends upon the current relative costs of the two processes.

Because of all the factors involved—stability of the drug in aqueous solution, effectiveness when administered orally, degree to which blood pressure is affected, compatibility with local anesthetics, volatility and individual idiosyncrasy—a wide selection of pressor agents would seem advantageous.

Diagnostic Agents.—Like so many other discoveries which have originated from purely academic research, that of phenolphthalein, made in 1880, has proved to be of considerable significance to medicine. Its importance as an indicator was recognized almost at once, but it was not until about twenty years later that its valuable laxative property was discovered. After another twenty

years had passed, it was found that a sulfur analog, phenolsulfonephthalein, is eliminated completely from a healthy individual, after intramuscular or intravenous injection, in a remarkably short time; delayed elimination is an indication of kidney dysfunction. Halogen derivatives of phenolphthalein—chloro, bromo, and iodo—are used for such purposes as roentgenologic examination of the gall bladder and as a test of liver function. Fluorescein, closely related to phenolphthalein in chemical structure, finds application as a diagnostic agent in ophthalmology.

Several iodated pyridine compounds are available for intravenous pyelography.

Since the use of the synthetic products discussed above has made exploratory surgical operations unnecessary in many instances, they represent discoveries which have been of very decided advantage to both the physician and the patient.

Diuretics and Laxatives.—During the search for antisypilitics among organic derivatives of mercury, it was discovered that certain representatives of this group are strong diuretics. Several of these mercury diuretics have already been used for a number of years. Merbaphen (novasurol) and salyrgan (mersalyl) are administered intravenously, and mercurin is administered rectally.

Laxatives which represent a single synthetic product of definitely established structure are very few in number. In addition to phenolphthalein, the oldest and best known member of this group, there is istizin (1, 8-dihydroxyanthraquinone) and isacen, an oxindole derivative.

Vasodilators.—Two outstanding effects produced by choline are a lowering of blood pressure and the promotion of gastric and intestinal peristalsis. It has been found that the activity of the compound is increased to a very great degree if certain structural changes are made in the molecule. Modifications of choline which have been found to be valuable drugs for a variety of conditions are acetylcholine chloride, acetyl- β -methylcholine chloride, doryl, and prostigmin.

Analgesics.—Although antipyrine, one of the oldest synthetic drugs, and aminopyrine (pyramidon) are very useful analgesics and antipyretics, they often fail to afford relief from severe pain. Consequently, the physician is still dependent on morphine and certain of its derivatives, the objectionable features of which are too well known to require mention. It seems very probable that if the carefully planned research program of a group of investigators in this country, begun about ten years ago, is continued, a satisfactory synthetic morphine substitute, free from such properties as tolerance and addiction, will be discovered.

The introduction of the synthetic product, dilaudid, indicates that some progress has already been made.

Stimulants for Circulation and Respiration.—There have been very interesting developments in the search for synthetic compounds which would be not only more reliable but which would lack the undesirable properties of naturally

occurring drugs such as strychnine, camphor, caffeine, epinephrine, and digitalis. Among the synthetic representatives in this group are nikethamide (coramine), homocamfin (cyclosal), metrazol (cardiazol), and neospiran.

Germicides.—One of the many difficulties encountered by the investigator searching for more active germicides is the lack of a quantitative method of evaluation by means of which their effectiveness can be determined under clinical conditions. Products which laboratory tests have shown to be of merit often fail to prove satisfactory when they are applied in medical practice.

The "universal germicide" seems to be as far from attainment as the philosopher's stone, and we have practically no knowledge with regard to the mechanism of germicidal activity. However, a definite advance has been made by the recognition that all germicides tend to be specific in their activity and that their ability to kill or retard the growth of microorganisms is dependent on a certain environment. To a considerable extent successful prophylaxis or treatment of an infection must depend upon the proper selection of a germicide and a recognition of the various factors involved in its use.

Organometallic compounds which contain mercury continue to find favor as bacteriostatic agents. Proprietary drugs of this type include mercurochrome, metaphen, meroxyl, mercurophen, merthiolate, sulfomerthiolate, merphenyl nitrate, and mercesin. Any opinion which may have been held that such products must be complex in structure in order to be effective has been disproved by the discovery of the activity of such simple compounds as basic phenylmercuric nitrate and 2-hydroxyphenylmercuric chloride.

A number of other organic derivatives of mercury find use as anti-syphilitics and diuretics.

Azochloramid is a product which belongs to the chloramine group, and zephiran is the first example of an organic germicide of the ammonium type.

Although it has been known for a long time that the phenol coefficient is increased to a great degree by the introduction of halogen or an alkyl group into a phenolic substance, many years passed before practical use was made of phenols which contained both halogen and alkyl radicals, for example, chlorothymol and a wide variety of other chloroalkylphenols.

The discovery that mandelic acid and sulfanilamide are effective germicides in the urinary tract is of great significance.

CHEMOTHERAPEUTIC AGENTS

Anthelmintics.—Since its introduction in 1921, carbon tetrachloride has been used to treat many millions of human beings for hookworm. In some respects tetrachloroethylene seems to be a better drug. Later, the use of hexylresoreinol was advocated and has been given extensive trial. Gentian violet medicinal, suggested for pinworms in 1938, has been used with considerable success. No effective chemotherapeutic agent is known for trichinosis or filariasis.

Trypanocides.—Three drugs of outstanding merit in the treatment of trypanosomiasis are tryparsamide, orsanine, and germanin (Bayer-205).

The complex urea derivative, germanin, introduced about 1920, produces most beneficial effects in African sleeping sickness. It is an example not only of a very brilliant achievement in the field of synthetic drugs, but also of one of the most baffling problems relative to the relationship between chemical structure and therapeutic activity. It is impossible to understand just how the presence of a methyl group can affect in any profound manner the physical, chemical, or pharmacologic properties of a molecule as large as that of germanin ($C_{51}H_{34}O_{23}N_6S_6Na_6$). Nevertheless, the removal of one of the two methyl radicals present in this substance or a shift in its position renders the product almost inactive.

Spirocheticides.—More than eight thousand organic derivatives of arsenic have been prepared but only about twelve of these have found extensive application as medicaments. To the short list of widely used spirocheticides—arsphenamine (salvarsan), neoarsphenamine, sulfarsphenamine, and silver arsphenamine—there has been added, recently, mapharsen. Although this latter substance was known many years ago as “arsenoxide,” its valuable therapeutic properties were not established until 1934.

The massive dose arsenotherapy of syphilis by the intravenous drip method, which has been introduced quite recently, has aroused great interest.

Antimalarial Compounds.—In spite of numerous investigations made in the hope that more effective drugs might be discovered, the quinoline compound plasmochin and the acridine derivative atabrin still remain the most valuable adjuvants to quinine in the treatment of malaria. Progress in this field is made especially difficult due to the fact that all known medicaments exhibit a selective action, that is, their effectiveness varies enormously, depending upon the species, strain, and phase of the parasite.

Drugs for Acute Bacterial Infections.—The efficacy of sulfanilamide, introduced in 1936, in infections due to streptococci, meningococci, and gonococci has been definitely established. In spite of the unparalleled success of this drug, thousands of other sulfur-containing products have been prepared and tested in the hope that new substances would be discovered which would lack its many undesirable by- and after-effects and by means of which the range of therapeutic usefulness could be extended. At present there seems to be little doubt about the value of sulfapyridine (M. and B. 693, Dagenan) in the treatment of pneumococcal infections; sulfathiazole seems to be of definite value for staphylococcal infections.

The forerunners of sulfanilamide, the red dye prontosil and the soluble dye prontosil soluble (neoprontosil), still find application.

Two arsenicals used extensively for the treatment of amoebiasis are ecarbarson and acetarson (stovarsol), while the antimony compound stibosan is employed to combat visceral leishmaniasis.

The elucidation of the structures of many vitamins and hormones and the discovery of processes by means of which they can be prepared in the laboratory, have opened a new field for synthetic work. In some instances, study of these structures has supplied clues which have enabled the chemist to prepare active products which are much simpler in structure than the naturally occurring

substances. It seems not improbable that in the future some of these synthetic products will find favor as therapeutic agents because of such factors as the relative ease with which they can be manufactured, greater stability, and certain unique properties not possessed by the natural vitamins and hormones. Possibilities in this direction are illustrated by compounds such as diethylstilbestrol, diiodothyronine, and certain analogs of vitamin K₁.

Of necessity, the selection of drugs discussed in this article is arbitrary. The mention of a product is not intended to imply recommendation nor does its omission denote lack of therapeutic merit.

REFERENCES

- May, Percy, and Dyson, G. M.: *The Chemistry of Synthetic Drugs*, New York, 1939, Longmans, Green & Co.
- Findlay, G. M.: *Recent Advances in Chemotherapy*, Philadelphia, 1939, P. Blakiston's Son & Co.
- Fischl, V., and Schlossberger, H.: *Handbook of Chemotherapy*. Part I, Metal-Free Organic Compounds, 1933; Part II, Metallic Compounds, Baltimore, 1934, H. G. Roebuck & Son.
- Green, Richard: *Lectures on the Development and Use of the Synthetic Anti-Malarial Drugs*, *Bulletins From the Institute for Medical Research, Federated Malay States*, No. 2, 1934.
- Field, J. W.: *Notes on the Chemotherapy of Malaria*, *Ibid.* No. 2, 1938.
- Mellon, R. R., Gross, P., and Cooper, F. B.: *Sulfanilamide Therapy of Bacterial Infections*, Springfield, Ill., 1938, C. C. Thomas.
- Long, Perrin H., and Bliss, Eleanor A.: *The Clinical and Experimental Use of Sulfanilamide, Sulfapyridine and Allied Compounds*, New York, 1939, The Macmillan Co.

THE SEROLOGY OF SYPHILIS*

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I. INTRODUCTION

THE serology of syphilis has a number of anomalies. It is interrelated with the immunology of syphilis, but it is also interrelated with biologic changes in the absence of syphilis. Serologic tests may employ spirochetal antigens, but they actually employ extracts of normal tissue as antigens. Serologic tests in infections other than syphilis are limited in the degree of sensitivity by the respective infections, as, for example, the agglutination test in typhoid fever, but serologic tests in syphilis may be so improvised as to show a degree of sensitivity not at all limited by this disease. Serologic tests in infections other than syphilis generally consist of but few technical diagnostic procedures, while the number of technical procedures of serodiagnostic tests for syphilis is legion. In addition, syphilis may be manifested by many clinical signs and symptoms occurring in other diseases or by no clinical signs and symptoms, a situation which often makes the serologic test the deciding factor in establishing the diagnosis.

The many varied aspects of the serology of syphilis and the importance of serologic tests in diagnosis make it understandable why we have evaluation committees and serologic surveys; why one laboratory employs one test and another perhaps five; why one believes that test A is better than test B and another, that test B is better than A; why the serology of syphilis, approaching forty years of existence, is still in as controversial a stage as it ever has been.

Yet, the serology of syphilis is beginning to enter a highly promising era. Evidence is accumulating that positive serologic reactions are divisible into two distinct types which can be differentiated in the laboratory: one type being a manifestation of syphilis and the other, unrelated to this disease. Perhaps in this differentiation lies the promise of making serologic tests true diagnostic agents in syphilis.

II. SEROLOGY IN RELATION TO IMMUNOLOGY OF SYPHILIS

Becoming Inoculated With the Treponema pallidum (Spirocheta pallida).—

It is well known that when man becomes inoculated with the *T. pallidum* he shows no signs or symptoms for a considerable period, extending from several weeks to several months. This situation is what might be expected. Man lacks immunity to the *T. pallidum*. To lack immunity to microorganisms is to lack the capability of localizing or fixing the microorganisms in the area in the skin or mucous membrane where they gain entry and of preventing them from invading the blood stream. Absence of this localizing capacity means absence

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of a local inflammatory reaction.¹ Also, the entry of microorganisms into the blood stream is apparently not disturbing to a nonimmune host. The result is that the invaded host is not cognizant of the infection.

In the case of the spirochetes of syphilis, the likelihood is that the non-specific localizing capability of the skin and mucous membrane for these microorganisms plays some role in the establishment of the infection. If the spirochetes are few in number and of low virulence, and this localizing capability is sufficient to hold them fixed in the area of entry, they may ultimately be destroyed locally. In most instances, while there may be some nonspecific localization of the spirochetes in the area of entry, this localization is undoubtedly insufficient to prevent them from finding their way into the blood stream. The localization in all probability leads to the formation of a disseminating focus of the spirochetes, a focus from which fresh microorganisms may enter the blood stream and replace those which are unable to survive in this medium. Disseminating foci are undoubtedly established also in the adjoining lymph glands. Thus do the spirochetes establish a strong foothold in the body.

As the spirochetes and their antigenic constituents come in contact with the tissue cells, they gradually bring about immune changes in them. Theoretically, we might expect to note the appearance of antibodies in about ten days or two weeks after the spirochetes gain entry into the body. These are the periods in which antibodies begin to be noted after the injection of an antigenic agent into an animal. In syphilis, however, the continued presence of the spirochetes in the blood stream may tend to delay antibody production. Generally, antibody production begins at a rapid rate as soon as the antigenic agent begins to disappear from the blood stream. Then again, antibodies produced at a time when the antigenic agent circulates in the blood stream may result in antibody-antigen union in vivo, thus reducing the detectable number of antibodies.

The Primary Lesion—A Manifestation of a Certain Degree of Immunity.—Absence of detectable antibodies in the blood stream does not of course mean absence of immunity. The first visible sign of the beginning of the development of immunity in syphilis is the primary lesion. This lesion is a localized one and, in the relationship between a host and microorganisms, a host must possess some specific immunity to keep a lesion produced by the microorganisms localized. Furthermore, in experiments with rabbits carried out in this laboratory in which protein antigens were employed,² it was possible to produce spontaneous ulcerative lesions in the skin, simulating the primary lesions of syphilis in human beings, only when the animals possessed a given degree of immunity.

These experiments can be carried out with relative ease. Rabbits are immunized to horse serum by weekly injections of, let us say, 0.1 c.c. per kilogram of body weight, administered by any route. A week after the fifth or sixth immunizing dose, the rabbits are injected intracutaneously with varying amounts of horse serum. These are so graduated as to cause local inflammatory reactions of varying intensity, as well as reactions so mild as to be invisible. To illustrate: 0.1 c.c. of undiluted serum and of 1:10, 1:100, 1:1,000, 1:10,000 and 1:100,000 dilutions of serum with salt solution are injected intracutaneously. Twenty-four hours later it may be found that the areas in which the undiluted

serum and the 1:10 and 1:100 dilutions had been injected show local inflammatory reactions of varying intensity, while the areas in which the 1:1,000, 1:10,000 and 1:100,000 dilutions had been injected appear normal.

The rabbits are now injected intravenously with a relative excess of horse serum, such as 20 c.c. or more. Soon after the intravenous injection it will be noted that the local skin areas in which the 0.1 c.c. quantities of the varying dilutions of horse serum had been introduced, show a distinct change. The three areas in which the undiluted serum and the 1:10 and 1:100 dilutions of the serum had been introduced and which show varying degrees of inflammation, first become purplish black, then black, especially in the centers of the inflammatory areas. The two areas in which the 1:1,000 and 1:10,000 dilutions of serum had been introduced and which showed no signs of inflammation, now also appear purplish black or black. The last area in which a 1:100,000 dilution of the serum had been introduced shows no change. Should the intravenous injections be repeated daily, the black areas will undergo visible tissue destruction and ulcer formation.

The degree of tissue necrotic flare-ups in preformed specific inflammatory areas in immunized animals, following the intravenous injection of antigen, varies with different rabbits. Some show marked tissue necrotic changes and some mild changes under the same experimental conditions.

In a broad sense, it may be said that tissue necrotic changes in a preformed specific inflammatory area, or in an area in which antigen had been injected in so small an amount as not to call forth visible inflammation, are dependent upon: (a) the degree of immunity of the host, (b) the amount of antigen introduced in the area, and (c) the concentration of the antigen in the blood stream. Similarly, it is believed that the formation of the primary lesion in syphilis (interpreted as a tissue necrotic change in a preformed invisible inflammatory area) is dependent upon the same three conditions. If any of these conditions do not conform to quantitative requirements, it is believed that chancre formation will not take place.

Serologic Reactions During Primary Stage.—The fact that antibodies are not detected by means of serodiagnostic tests at the time of the appearance of the chancre is of interest. In the case of the protein-immunized rabbits considered, a high precipitin titer was shown by them previous to the intravenous injection. Immediately following this injection, the precipitin titer became negative, indicating the disappearance of the antibodies from the circulation. At the time when the rabbits began to show the necrotic lesions within the preformed inflammatory areas, the titer was still either negative or very low. This relative absence of antibodies is believed to be due to antigen-antibody union *in vivo*.

It is likely that the absence of antibodies during the early primary stage is also due to antigen-antibody union *in vivo*. The appearance of the chancre is dependent upon a relatively high concentration of spirochetal antigen in the blood stream. In the presence of a high concentration of antigen, any antibodies that might be present might combine with the antigen *in vivo*, thus leading to seronegative reactions.

The intensity of the serologic reaction in primary syphilis may thus be dependent upon the relationship between the quantities of spirochetal antigen and of antibody present in the blood stream. In the early primary stage, the quantity of antigen probably exceeds the quantity of antibody in the blood stream, while in the later primary stage, the quantity of antibody undoubtedly exceeds the quantity of antigen. In the later stage, one notes definite evidence of immunity of the host against the spirochetes. The primary ulcer had teemed with spirochetes only a short while previously. Now spirochetes are difficult to find and the ulcer is undergoing spontaneous healing. This fact must mean that the host is developing substances destructive to the spirochetes; that it is gaining the upper hand, so to speak, over these microorganisms. Hence, an increase in the antibody titer at this time is to be expected.

It was stated that one of the requirements for the formation of the primary lesion is the presence of spirochetal antigen in sufficient concentration in the blood stream. The question may arise as to what is the relationship between the presence of antigen in the blood stream and the change to tissue necrosis in a preformed specific inflammatory lesion. The probability is that the circulating antigen is attracted to the inflammatory lesion. The basis of this attraction is not fully understood. It is known that a circulating dye tends to accumulate in a nonspecific inflammatory lesion.³ The likelihood is that circulating antigen will accumulate in a specific inflammatory lesion to a far greater extent than a dye. Inflammatory tissue probably possesses marked anchoring or localizing powers for specific antigen. If this view is accepted, then circulating spirochetes would be drawn to the focus of their entry which, although invisible to the naked eye, must be inflammatory. As the spirochetes accumulate in this focus the mild inflammatory reaction changes to a tissue necrotic reaction. This change is based on a principle in immunity emphasized elsewhere;² namely, when free antigen accumulates in a specific inflammatory area, this area undergoes tissue necrosis. Free antigen seems to interfere with the inflammatory process, the blood vessels apparently become filled with thrombi, and with the cutting off of the blood supply, death of tissue is the natural sequel.

Serologic Reactions During Secondary Stage.—Turning to the secondary stage of syphilis, the relationship between the serology and immunology of this stage is also of interest. Serologic reactions are almost invariably positive during this stage. The reason for this, in our opinion, lies in the role of the skin and mucous membranes in immunity. It was shown in our laboratory⁴ that an outstanding feature of the response of the tissues in immunity is the fact that the skin possesses a far greater attractive force for specific antigen than do other tissues. In the case of rabbits immunized with protein antigens, it was found that the skin possesses an anchoring or localizing power for the antigen from ten to fifteen times greater than that possessed by some of the other tissues, such as skeletal muscle. Unpublished experiments carried out by Stellwagen⁵ in our hospital indicate that the surface mucous membranes also possess this high localizing power for antigen. It is, therefore, likely that, as the immunity of the host approaches a given height at the beginning

of the secondary stage, the immunity of the skin and mucous membranes becomes highly intensified. The cutaneous tissue thus possessing a particularly marked attractive force for the spirochetes at this time, it might be expected that, as these microorganisms circulate in the blood stream, they are localized in large numbers in this tissue. This localization would in turn cause many spirochetes to be withdrawn from the circulation—a step which would result in an increase in the antibody titer and in positive serologic reactions.

Serologic Reactions During Other Stages of Syphilis.—In a broad sense, there are three situations in syphilis in which serologic reactions might be negative: (a) when antibodies are not yet formed, (b) when there is an excess of spirochetal antigen in the circulation, and (c) when spirochetal activity has become so quiescent as not to stimulate antibody production.

Little need be said about negative serologic reactions during the incubation period in syphilis before the formation of antibodies. All infections have a similar seronegative period. Negative serologic reactions resulting from an excess of spirochetes or spirochetal antigen in the circulation are in all probability not uncommon in syphilis. Such reactions during the early primary stage, already considered, are assumed to be due to an excess of circulating spirochetal antigen. These reactions are common in precocious malignant syphilis in which the host is literally overwhelmed with spirochetes. Indeed, it can be stated as a principle in immunity that antibody production is disturbed when a host is overwhelmed with an antigenic agent.

Negative reactions may also occur in marked syphilitic flare-ups when large quantities of spirochetal antigen are suddenly thrown into circulation. A syphilitic flare-up generally tends to stimulate antibody production, but the presence of spirochetal antigen in the circulation may lead to antigen-antibody union *in vivo*. When, therefore, the amount of antigen in the circulation is excessive, the antibodies may temporarily disappear from the circulation, undoubtedly due to antigen-antibody union.

Negative serologic reactions during spirochetal quiescence are also what might be expected. Antibody production depends on the antigenic stimulus of the body cells. As the antigenic stimulus is removed, the production of antibodies ceases. Antibodies will continue to circulate in the blood stream for days or weeks after their production has ceased. Sooner or later, however, serologic reactions are bound to become negative as a result of spirochetal quiescence.

Now and then a patient may give negative serologic reactions in the presence of old syphilitic lesions. This situation may be met with when spirochetal activity and destruction of tissue had occurred some time previously. To illustrate: A patient has an aortic aneurysm of syphilitic origin. The processes which led to the formation of the aneurysm may be arbitrarily divided into two steps: (1) an attack of syphilitic aortitis, during which the elastic tissue within the inflammatory area of the aortic wall was destroyed and replaced by fibrous tissue, and (2) the slow and steady expansion of the

fibrous area into a tumorlike mass. It is clear that while the spirochetes had been active during the first step of aneurysm formation, they are not necessarily active during the second step. Inactivity, in turn, means little or no liberation of spirochetal antigen and, hence, little or no stimulus to antibody production. The same statement applies to a perforated nasal septum and to other old destructive processes of syphilitic origin.

One also encounters in syphilis, especially during therapy, fluctuating serologic reactions. A serum may be positive on a given day and but weakly positive or doubtful several days later or vice versa. Such results are naturally annoying to physicians. The fact, however, is that neither negative reacting sera nor strongly positive sera show a tendency toward marked fluctuations. Only sera giving borderline positive results may show this tendency, which might be due to the colloidal-chemical nature of the serologic tests employed. An outstanding feature of colloidal-chemical methods is that small variations in technique often lead to apparently marked changes in the results.

It is undoubtedly because serologic tests are based on colloidal surface reactions that we are faced with such large numbers of tests. The differences between the various precipitation tests are very much like the differences in the formula for baking a chocolate cake. The ingredients are very much the same, but the methods of mixing them are extremely important if one wants a really good cake. The same applies to the differences between the various complement fixation tests.

Since it is best that human ingenuity be stimulated rather than disturbed, it is just as well to be faced with the problem of increasing numbers of "new" serologic tests. Perhaps some day the perfect test will make its appearance. Even then, the problem of its performance by imperfect human hands will still remain.

In summarizing, we have seen that serology is intimately bound up with the immunology of syphilis; that serologic reactions in syphilis behave essentially like serologic reactions in other infectious diseases; that the same laws which govern serologic reactions in immunity apparently govern serologic reactions in syphilis; that all indications are that serologic reactions in syphilis are specific immune reactions.

III. SEROLOGY IN RELATION TO NONSYPHILITIC CONDITIONS

The serology of syphilis in relation to nonsyphilitic conditions presents a picture quite different from that just considered. One finds that serology is not merely a part of the immunology of a given infectious disease and serologic reactions are not necessarily associated with syphilis. Serology becomes a part of a biologic phenomenon not at all limited to infection and serologic reactions appear under multiple conditions in human beings and lower animals in the absence of syphilis. Let us consider these nonsyphilitic serologic reactions.

Ultramicroscopic Reaction Between Nonsyphilitic Sera and Tissue Extract Antigen.—Ultramicroscopic precipitation studies carried out in this laboratory⁴ showed that the type of antibody responsible for positive serologic reactions in

syphilis is widely distributed among human beings in the absence of syphilis. According to these studies, of a given number of sera giving negative serologic reactions, approximately one-third will show some precipitation with extract antigen when examined with the ultramicroscope. Table I gives the precipitation results obtained with 50 nonsyphilitic sera. It is evident that 17 of the sera showed a definite tendency toward precipitation with extract antigen. In this experiment the precipitation property of nonsyphilitic sera becomes evident only under conditions of high magnification. In the following experiment this precipitation property becomes evident without magnification.

TABLE I

SIZE OF AGGREGATES FORMED WHEN STANDARD ANTIGEN SUSPENSION (KAHN) IS MIXED WITH SERA FROM NONSYPHILITIC AND SYPHILITIC PERSONS AND EXAMINED ULTRAMICROSCOPICALLY

NUMBER OF SERA	CROSS-SECTIONAL AREA OF AGGREGATES IN SQUARE MICRONS	
	RESULTS READ	
	AFTER 1 HOUR	AFTER 6 HOURS
Nonsyphilitic		
11	0	0
13	0	10- 20
9	0- 10	20- 100
7	10- 50	100- 350
5	50- 250	350- 500
5	250- 500	500- 1,500
Syphilitic		
2	250- 500	500- 600
9	250- 500	600- 1,500
24	500- 3,000	1,500- 4,000
12	500- 3,000	4,000-10,000
3	3,000-10,000	10,000-25,000

Precipitation Property of Nonsyphilitic Sera With Highly Sensitive Antigens.—In the course of routine standardization of antigen in this laboratory during the past decade, antigens have been encountered many times more sensitive than standard Kahn antigen. Thus, in the University Hospital where serologic examinations are made routinely on all admissions, the number of positive reactions ranges between 3 and 4 per cent. With the especially sensitive antigens, however, the number of positive reactions in the same cases may exceed 30 per cent (Tables II and III).

TABLE II

SENSITIVITY OF STANDARD KAHN ANTIGEN COMPARED WITH THAT OF AN EXCESSIVELY SENSITIVE ANTIGEN*

Observed in routine standardization of antigen at the Serological Laboratory of the University of Michigan Hospital.

NUMBER OF SERA	STANDARD KAHN ANTIGEN	EXCESSIVELY SENSITIVE ANTIGEN
3	Positive	Positive
1	Doubtful	Positive
7	Negative	Positive
5	Negative	Doubtful
4	Negative	Negative

*Standard antigen lot 18; excessively sensitive antigen lot CN.

TABLE III

SEROLOGIC RESULTS GIVEN BY AN EXCESSIVELY SENSITIVE ANTIGEN COMPARED WITH THOSE GIVEN BY THE KAHN STANDARD AND PRESUMPTIVE ANTIGENS

Sera (504) taken from general run of hospital admissions.

ANTIGEN	POSITIVE	DOUBTFUL	NEGATIVE
Standard	15	3	486
Presumptive	20	2	482
Excessively sensitive	137	84	283

Serologic Reactions of Nonsyphilitic Sera From Patients With Leprosy and Malaria.—That serologic tests give positive reactions with the sera of patients with leprosy and malaria in the absence of syphilis is well known. Yet, it seemed worth while to summarize the serologic results obtained in these two diseases with thirteen different complement fixation and precipitation tests.⁷ It is evident from Table IV that leprosy patients give from 42 to 76 per cent positive serologic reactions and malaria patients, from 8 to 19 per cent positive reactions with different tests.

TABLE IV

FALSE POSITIVES REPORTED BY 13 DIFFERENT SEROLOGISTS ON BLOOD SPECIMENS FROM PRESUMABLY NONSYPHILITIC PATIENTS WITH LEPROSY AND MALARIA

(From Supp. No. 1, Ven. Dis. Infor. Washington, D. C., 1935.)

	LEPROSY—50 CASES	MALARIA—36 CASES
	PERCENTAGE OF FALSE POSITIVES	PERCENTAGE OF FALSE POSITIVES
Brem*	44	14.3
Eagle	72	12.1
Hinton	40	11.1
Johns	58	11.4
Kahn	60	11.4
Kline	66	14.3
Kolmer*	64	19.4
Kurtz†	76	16.7
Lufkin and Rytz	70	11.1
Rein†	68	19.4
Ruediger*	62	20.6
Weiss	52	8.6
Williams*	42	17.1

*Performed modification of complement fixation tests. All others performed flocculation tests.

†Performed Kahn presumptive tests.

‡Performed Kline exclusion tests.

Nonsyphilitic Conditions in Which Positive Serologic Reactions May Be Obtained.—It is generally assumed, based on textbook reports, that false positive reactions may be obtained only in isolated pathologic conditions (in addition to leprosy and malaria), such as in infectious mononucleosis and malignancy. A study of the reports of the serologic evaluation studies since 1928 reveals, however, that false positives may be obtained in many pathologic conditions and also now and then under apparently normal conditions. The reports of the League of Nations Health Organization of the Copenhagen (1928)⁸ and Montevideo (1930)⁹ serologic conferences list among the conditions in which false positives have been obtained: acute bronchitis, psoriasis, acute cerebral hemorrhage, dementia praecox, epilepsy, senile cachexia, measles

headache, and many others. It might be added that the pathologic conditions in which false positives were obtained in the American Evaluation study of 1934-1935⁷ include, aside from leprosy and malaria, tuberculosis, malignant neoplastic disease, acute febrile diseases, and jaundice. False positives occurred also in pregnancy, during menstruation, and in other apparently normal conditions.

Positive Serologic Reactions in Lower Animals in the Absence of Syphilis.—It is well known that many of the lower animals give positive serologic reactions. It does not matter what particular method one employs, whether a complement fixation or a precipitation test, the reactions with the sera of horses, cows, pigs, chickens, and other animals are positive in most instances. Table V illustrates the relative frequency of positive serologic reactions given by lower animals. Table VI shows that lower animals may give positive reactions of relatively high quantitative titers.

TABLE V
POSITIVE KAHN REACTIONS IN NORMAL ANIMALS

ANIMAL	NUMBER OF BLOOD SERA TESTED	POSITIVE REACTIONS	NEGATIVE REACTIONS
Rabbit	29	21	8
Horse	21	19	2
Hog	42	42	0
Sheep	10	10	0
Cow	7	7	0
Calf	4	0	4
Dog	9	1	8
Chicken	54	32	22

TABLE VI
QUANTITATIVE KAHN REACTIONS IN NORMAL ANIMALS

ANIMAL	NUMBER OF BLOOD SERA TESTED	SEROLOGIC TITER
Rabbit	6	4
	5	20-40
Sheep	4	20-40
	1	80
Hog	10	40-80
	10	120-200
Cow	6	40-80
	1	160

Are Serologic Reactions With Spirochetal Antigens More Specific Than With Tissue Extract Antigens?—Beck,¹⁰ in a recent comparative study of spirochetal and tissue extract antigens in complement fixation tests, found the two antigens of similar specificity. In 496 nonsyphilitic sera, he reported 1.6 per cent doubtful or positive reactions with a spirochetal antigen and 1.2 per cent with a tissue extract antigen. More recently, Eagle and Hogan¹¹ and Erickson and Eagle¹² have also made comparative studies of the two types of antigens and their results are definitely in favor of the spirochetal antigen (Reiter strain). Out of a group of 540 hospital cases which gave negative flocculation and complement fixation (Eagle) reactions, 17 gave reactions with

spirochetal antigen. Of these, 14 were accompanied by a history of syphilis, and only three were probably nonspecific.

These investigators also consider the nature of the antigen and antibody in the serologic tests with tissue extract and spirochetal antigens. According to Beck, the spirochetes contain a specific antigen and an "ubiquitous lipid substance," evidently common in mammalian tissue. Syphilitic serum contains two different antibodies: one reacting with the specific antigen in the spirochetes and the other with the lipid antigen. Beck demonstrated by means of absorption tests three corresponding antibodies for three serologically different types of spirochetes (Reiter, Kroo, and Noguchi). Eagle and his associates find that the absorption of syphilitic serum with spirochetal suspensions renders the serum serologically negative to both spirochetal and tissue extract antigens. On the other hand, the absorption of syphilitic serum with tissue extract renders the serum serologically negative to tissue extract antigen but not to spirochetal antigen. These authors conclude that spirochetal cultures (Reiter) contain antigenic material serologically related to a substance present in mammalian tissue, as well as other antigenic factors not present in such tissue. To quote Erickson and Eagle: "Since suspensions of cultured spirochetes contain antigenic factors which react specifically with syphilitic serum, some of which are not present in ordinary Wassermann and flocculation antigens, they may prove even more valuable than tissue extracts in the serodiagnosis of syphilis."

Returning to the question of specificity of spirochetal antigens, one might expect that the use of such antigens would lead to results very nearly completely specific for syphilis. For example, in the absence of syphilis in leprosy and in malaria, the results with such antigens should be negative. Yet, a serologic study of leprosy by means of the complement fixation test with spirochetal antigen by Cappelli,¹³ indicates that the use of this antigen does not assure absolute specificity. Of 23 nonsyphilitic leprosy patients studied, Cappelli reported 18 one-plus or two-plus reactions on the first reading, and 5 one-plus reactions on the second reading. It is thus evident that nonsyphilitic leprosy patients give at least weak or moderate complement fixation reactions with spirochetal antigen.

In the case of complement fixation tests with spirochetal antigen in malaria, in the absence of syphilis, Eagle¹⁴ has pointed out that the results, so far as specificity is concerned, are similar to those given by complement fixation and flocculation tests with tissue extract antigens. It is difficult to explain why serologic tests with spirochetal antigen should give false positive reactions in malaria. Perhaps it is the "ubiquitous lipid substance" of Beck present in the bodies of the spirochetes that is responsible for the positive reactions both in malaria and leprosy. Eagle also claims that spirochetal suspensions contain antigenic material "related to a substance present in mammalian tissue." Thus one must conclude that, based on present studies, spirochetal antigens, although apparently somewhat more sensitive than tissue extract antigens used in diagnostic tests, are no more specific than these antigens.

Why False Positives?—The positive serologic reactions in syphilis are not difficult to explain. We have already seen that in all probability they are intimately related with immunity in syphilis. If we accept the point of view that tissue extracts have something in common with the antigenic structure of the spirochetes, the immune nature of serologic reactions becomes further understandable. However, none of these views throws light on the basis of serologic reactions in the absence of syphilis. If we would meet with false positives only in malaria and leprosy, the problem would not seem so complex. But such reactions are met with in any type of pathologic condition and in the apparent absence of such condition. In addition, many of the lower animals give false reactions.

Elsewhere¹⁵ I have stated it is conceivable that the basis for these serologic reactions (in lower animals) is the likelihood that the animals are carriers of nonpathogenic spirochetes. Man, for example, is known to give low titer agglutinin reactions to typhoid bacilli without regard to typhoid infection or vaccine immunization. The explanation is that the colon and typhoid bacilli possess certain antigenic constituents in common, and man, being a carrier of colon bacilli, possesses some natural antibodies against typhoid bacilli. On the same basis it might be assumed that lower animals possess natural antibodies against spirochetes.

However, even if there is some basis of fact in this explanation of positive reactions in animals, it apparently is not applicable to false positives given by man. For these positives generally correspond to some pathologic disturbance. A patient with jaundice, for example, might give a positive reaction, but on recovery, this reaction generally becomes negative.

There is still another aspect with regard to false positives; namely, the type of test employed. Recognizing that traces of *reagin* commonly occur in sera of human beings, it is understandable why the sensitivity of a test is so interrelated with its specificity. Thus, when employing a dependable diagnostic test, sera containing minute amounts of antibody are likely to give reactions that are either negative or so close to the negative level as to be reported negative. If the sensitivity of a test is increased beyond proper limits, traces of antibody may be sufficient to give definitely positive reactions. Hence, highly sensitive methods are likely to give comparatively large numbers of false positives.

It should be stated that we have not been concerned here with technical errors in the performance of serologic tests leading to false positives. Our concern has been the so-called biologic false positives and, to our knowledge, there is no acceptable explanation for their existence.

To summarize, experimental evidence is presented that the type of reaction which occurs in the serology of syphilis is not limited by this disease. By means of ultramicroscopic studies and by the use of excessively sensitive antigens, it can be shown that relatively large numbers of nonsyphilitic individuals may give positive reactions. It is also well known that nonsyphilitic individuals with various pathologic conditions may give positive reactions with routine diagnostic tests. The same tests also give positive reactions

with the sera of different animals in the absence of syphilis. It is thus evident that serologic reactions in the absence of syphilis are not isolated and unique, but they represent a distinct and widespread biologic phenomenon.

IV. DIFFERENTIATION BETWEEN SEROLOGIC REACTIONS IN SYPHILIS AND IN THE ABSENCE OF SYPHILIS

Assuming that it were possible to distinguish serologic reactions occurring in syphilis from those occurring in the absence of this disease, serology would be placed on a new scientific foundation. The serology in syphilis and in the absence of syphilis would then become distinct from one another. The serology in syphilis would assume new specificity, hence, new dependability; and the serology in the absence of syphilis would lend itself to special studies as to its *raison d'être*. These were the thoughts that for many years kept studies on the detection of false positives alive in this laboratory, but only recently did these studies begin to show promise.

Strange as it may seem, the basis for the detection of false positives lies within the very serologic procedures in common use. The factor which differentiates true from false positives is the temperature at which these serologic procedures are carried out.

It is well known that the temperature at which serologic tests are performed markedly affects the final results. In the case of the standard Kahn test which is performed at room temperature, it has been observed again and again that high room temperature and low room temperature do not lead to the same results. Generally, temperatures considerably above 21° C. lead to increased sensitivity, and temperatures considerably below 21° C. lead to decreased sensitivity. But these observations apparently apply only to syphilitic sera.

Studies of the effect of temperature on the Kahn reaction with animal sera were found to present a different picture. These sera tend to give more marked precipitation at temperatures below 21° C. than at temperatures above 21° C.

This difference in the response to temperature by seropositive syphilitic and animal sera formed the basis of studies which led to the development of a procedure for the detection of false positive reactions in man. This procedure consists in performing the standard Kahn test at 37° and 1° C., respectively. The technique is described elsewhere.¹⁶ Herewith, we shall limit ourselves to brief considerations of several observations brought to light by this differential temperature procedure.

Observation 1. Serologically positive sera of lower animals react more strongly at 1° than at 37° C. Serologically positive sera from horses, cows, pigs, rabbits, and other animals, when examined with the standard Kahn test, carried out at 37° and 1° C., respectively, give stronger precipitation reactions at 1° than at 37° C. The types of reactions obtained at these two temperatures depend on the serologic potency of the sera. Some sera are moderately positive in the cold and negative at 37° C.; some are strongly positive in the cold and negative or moderately positive at 37° C.; those which are so potent as to be strongly positive at both temperatures can be rendered positive in the cold and negative at 37° C. at given dilutions of the serum with salt solution.

Observation 2. Serologically negative sera of lower animals are either negative at 37° and at 1° C., or are negative at 37° and positive at 1° C. The tendency of animal sera to give precipitation reactions in the cold is not limited to those sera which are positive with a serodiagnostic test. The same tendency is noted also with sera which are negative with a serodiagnostic test. When such sera are serologically examined at 37° and 1° C., approximately one-half will be found to give positive precipitation reactions at 1° and negative reactions at 37° C.; the remainder are negative at both temperatures.

Observation 3. Serologically positive syphilitic sera react more strongly at 37° than at 1° C. When syphilitic sera giving positive reactions with a serodiagnostic test are examined serologically at 37° and 1° C., precipitation will be found to be marked at 37° and weak or negative at 1° C. In the case of syphilitic sera having a high serologic titer, the reactions at 37° and 1° C. may be similarly positive. If such sera are first diluted with salt solution to a degree that they give weakly positive reactions with a diagnostic test, and are then examined at the differential temperatures, precipitation will be marked at 37° and weak or negative at 1° C. As will be seen below, the behavior of syphilitic sera at the differential temperatures is not as consistent as the behavior of animal sera at these temperatures.

Observation 4. Some serologically negative syphilitic sera are positive at 37° and negative at 1° C. When serologically negative sera from treated cases of syphilis are examined at 37° and 1° C., some will be found to give a positive precipitation reaction at 37° and no reaction at 1° C. Now and then a serologically negative serum will show similarly weak precipitation at both of the differential temperatures.

Observation 5. Serologically positive nonsyphilitic sera react more strongly at 1° than at 37° C. (a) *Malarial Sera.* An opportunity was afforded us to study the serologic behavior of malarial sera at the differential temperatures. The study was undertaken by Dr. L. E. Burney of the United States Public Health Service and Dr. J. R. S. Mays, Assistant Physician of the Milledgeville State Hospital, Milledgeville, Ga. Various syphilis-free patients in this hospital were treated by inoculation with malaria, and the serologic reactions were studied before, during, and after the malarial paroxysms. A number of these patients gave positive serologic reactions with diagnostic tests. When testing the serologically positive sera of these patients at the differential temperatures, they showed stronger precipitation reactions at 1° than at 37° C.

(b) *Leprosy Sera.* Some serologically positive leprosy sera show stronger reactions at 37° than at 1° C., and others show stronger reactions at 1° than at 37° C. It is likely that the former sera are from patients who have syphilis in addition to leprosy, and the latter are from patients who have only leprosy. In view of the fact, however, that it is extremely difficult to differentiate clinically syphilis from leprosy in many instances, the interpretation of these serologic findings at the differential temperatures must be held in abeyance.

(c) *Miscellaneous Sera.* Serologically positive sera in which the reactions are believed to be false are frequently being received in this laboratory for examination from all over the United States. Many of these sera show strong

precipitation at 1° and negative precipitation at 37° C., and when the clinical histories of these cases are sent to this laboratory, all indications point to the absence of syphilis. Occasionally, this type of precipitation is obtained with a serum from a febrile patient giving a positive reaction with a serodiagnostic test during the febrile period, but a negative reaction when the fever subsides. Recently a specimen of blood from a 17-year-old boy was examined at the differential temperatures. The boy won a scholarship in one of our leading institutions of learning and was clinically free from any suggestion of syphilis. The finding of a stronger precipitation at 1° than at 37° C. with this boy's serum led us to report a nonsyphilitic type of reaction.

Observation 6. Serologically negative nonsyphilitic sera are either negative at 37° and at 1° C., or are negative at 37° and positive at 1° C. Serologically negative nonsyphilitic sera might be expected to be negative also at 37° and 1° C. Actually, many of these sera give precipitation reactions at 1° C. The number of these reactions in the cold depends on the source of the sera. In the case of normal persons these reactions may not exceed 2 per cent. In hospital cases they may reach 4 per cent or higher. In leprosy and malaria these reactions in the cold have been found to reach as high as 50 per cent. Table VII summarizes Observations 1 to 6 in outline form.

TABLE VII

RESULTS GIVEN BY THE DIFFERENTIAL TEMPERATURE TECHNIQUE (VERIFICATION TEST)
WITH VARIOUS SERA

SERUM NUMBER	REACTION OF SERODIAGNOSTIC TEST	PRECIPITATION RESULTS:					
		37° C.			1° C.		
		Tube 1	Tube 2	Tube 3	Tube 1	Tube 2	Tube 3
Serologically positive sera of nonsyphilitic lower animals							
1	Positive	-	-	-	++++	++++	++++
2	Positive	-	++	+++	+	++++	++++
3	Doubtful	-	±	++	-	++	++++
Serologically negative sera of nonsyphilitic lower animals							
1	Negative	-	-	-	-	-	-
2	Negative	-	-	-	++++	++++	++++
3	Negative	-	-	-	-	+	++
Serologically positive sera of syphilitic human beings							
1	Positive	++++	++++	++++	-	-	-
2	Positive	±	++++	++++	-	+	++++
3	Doubtful	-	±	++++	-	-	±
Serologically negative sera of syphilitic human beings							
1	Negative	-	-	-	-	-	-
2	Negative	-	++	++++	-	-	-
3	Negative	-	±	++	-	-	-
Serologically positive sera of nonsyphilitic human beings							
1	Positive	-	-	-	++++	++++	++++
2	Positive	-	++	+++	+	++++	++++
3	Doubtful	-	±	++	-	++	++++
Serologically negative sera of nonsyphilitic human beings							
1	Negative	-	-	-	-	-	-
2	Negative	-	-	-	++++	++++	++++
3	Negative	-	-	-	-	+	+++

Observation 7. Exceptions. The foregoing observations indicate that positive serologic reactions given by serodiagnostic tests are divisible into two broad types: a syphilitic type and a nonsyphilitic type. Sera giving the former

type of reaction tend to give stronger serologic results at 37° than at 1° C., while sera giving the latter type of reaction, tend to give stronger serologic results at 1° than at 37° C. In view of the fact that the nonsyphilitic type of reaction is encountered under various conditions in man and is relatively widespread among lower animals, it is referred to as the general biologic type of reaction.

But clear-cut syphilitic and general biologic types of serologic reactions are not the only types encountered in practice. Serologically positive human sera may show both types of reactions. Considering the widespread distribution of the general biologic type of reaction in the absence of syphilis, there is no reason why it should not also be found in the presence of syphilis. Hence, it might be expected that certain cases of syphilis might give at the same time both the syphilitic and the general biologic types of reactions.

Then again, the general biologic type of reaction may occur in syphilis; as has been observed in this laboratory in cases of neurosyphilis treated with malaria. A patient may show a positive spinal fluid reaction and a weakly positive or negative serum reaction. During malarial therapy he may begin to give strongly positive serum reactions with a diagnostic test. On examination of the serum at 37° and 1° C., it is found that the reactions are strongly positive at 1° and weakly positive or negative at 37° C., indicating that these reactions are not related to syphilis, but to the malaria therapy.

In those instances in which the results of the differential temperature technique are inconclusive, certain supplementary procedures may prove of assistance. A procedure most frequently employed is based on the observation that sera giving borderline positive serologic reactions of a nonsyphilitic nature tend to show precipitation not only at 1° C. after they have been heated for thirty minutes at 56° C., but also in an unheated state. Syphilitic sera giving borderline positive serologic reactions differ in this respect. These weakly positive sera give little or no precipitation in the cold after they have been heated at 56° C., and they are especially likely to give no precipitation in the cold when tested in an unheated state. Hence, when the results of the differential technique are inconclusive, a supplementary test is carried out with serum in an unheated state. If precipitation is obtained at 1° and no precipitation at 37° C., the probability is that the serologic reaction is of a nonsyphilitic type. The use of sensitized antigen (employed in the Kahn presumptive test) has also thrown light on a number of inconclusive types of reactions obtained with standard antigen. Studies of supplementary procedures are in progress with the aim of reducing to a minimum the number of inconclusive types of reactions with the differential temperature technique.

The Verification Test.—The differential temperature technique is referred to as the verification test. The value of this test is briefly twofold: 1. As a supplementary procedure for serodiagnostic tests whenever there is a question of false positives. 2. As a procedure for the study of the nature and significance of positive serologic reactions in the absence of syphilis.

The verification test as a supplementary serodiagnostic procedure. Whenever there is a question of the specificity of a positive reaction given by a

diagnostic test, the serum is examined with the verification test. Three types of reactions are likely with this serum:

1. Syphilitic type.
2. General biologic type.
3. Inconclusive type.

When sera giving negative reactions with a diagnostic test are examined with the verification test, four types of reactions may be noted.

1. Negative type (when no precipitation occurs at either 37° or at 1° C.).
2. General biologic type.
3. Syphilitic type (generally in treated cases of syphilis).
4. Inconclusive type.

The verification test in the study of the nature and significance of non-syphilitic serologic reactions. False positive serologic reactions are generally considered in relation to syphilis only. Once it is established that a given reaction is a false positive, the matter is generally dismissed. The question may arise, why it is that false positives in man are almost invariably associated with pathologic disturbances. In leprosy, malaria, and infectious mononucleosis these general biologic types of serologic reactions are encountered more frequently than in other pathologic conditions. Also, why it is quite rare to encounter these types of reactions in the absence of pathologic conditions. The fact that the general biologic types of reactions are encountered among serologically negative persons is also of great interest. Can it be said that the general biologic type of reaction in man is a manifestation of some disturbance, or do certain persons have the tendency to give this type of serologic reaction without regard to any disturbance?

V. SUMMARY—THE NEW SEROLOGY OF SYPHILIS

Indications are that the serology of syphilis, as it has developed during the past three decades, embraces two distinct types of positive reactions. One is essentially an immune serum reaction, similar to agglutination, precipitation, and other antigen-antibody reactions in immunity, which apparently is specifically associated with syphilis. The other type of reaction may or may not be associated with immunity; in lower animals it occurs under apparently normal conditions; in human beings it most frequently occurs under pathologic conditions, but it is definitely not associated with syphilis.

In the new serology of syphilis, every positive reaction in which there is a question as to its relationship to syphilis will be subjected to special studies, not alone with the verification test but also, should it prove necessary, with all possible supplementary procedures. The serologist then will assume full responsibility for his report just as the roentgenologist, for example, is responsible for his.

Should the serologic reaction be found to be of the general biologic type and unrelated to syphilis, clinicians will not disregard it, but will investigate the reason for this reaction, especially as to whether some pathologic condition is responsible for it. Repeated serologic studies might reveal an increase or a decrease in the potency of the reaction, and these changes might correspond to

changes in the pathologic condition. Then again, the studies might reveal, in some cases, a tendency toward positive serologic reactions in the apparent absence of pathologic conditions.

The new serology of syphilis will thus lead not only to more dependable laboratory diagnosis in syphilis, but also to the study of the significance of those reactions not associated with this disease. A positive serologic reaction in the diagnosis of an asymptomatic case will first of all require the "typing" of the reaction to determine whether it is syphilitic or general biologic. This determination may necessitate repeated serologic studies, considering that a person who shows no signs or symptoms of syphilis is to be diagnosed as syphilitic or as free from syphilis. The increased dependability of the serologic diagnosis of syphilis should remove objections against routine blood testing. This step, in turn, should greatly aid the control of syphilis.

REFERENCES

1. Kahn, R. L.: Tissue Immunity, Springfield, Ill., 1936, Charles C. Thomas, pp. 93-119.
2. *Ibid.*, pp. 285-312.
3. Menkin, V.: Studies on Inflammation. The Mechanism of Fixation by the Inflammatory Reaction, *J. Exper. Med.* 53: 171, 1931. See also Le Duc, Don M.: The Use of Intravenous Dye to Demonstrate Evidence of Inflammation in the Skin of Experimental Animals Following the Application of Irritating Substances, *Univ. Hosp. Bull., Ann Arbor* 5: 34, 1939.
4. Kahn, R. L.: Tissue Immunity, pp. 180-213.
5. Stellwagen, W. J.: Personal communication.
6. Malloy, A. M., and Kahn, R. L.: The Ultramicroscopic Precipitation Reaction in Syphilis, *J. Infect. Dis.* 48: 243, 1931.
7. The Evaluation of Serodiagnostic Tests for Syphilis in the United States, Supp. No. 1 to Ven. Dis. Inform., Washington, D. C., 1935, U. S. Government Printing Office, pp. 9, 10.
8. Report of Second Laboratory Conference on Serodiagnosis of Syphilis, Geneva, League of Nations Health Organization, 1928.
9. Report of Laboratory Conference on Serodiagnosis of Syphilis Convened at Montevideo, Geneva, League of Nations Health Organization, 1931.
10. Beck, A.: The Role of the Spirochaete in the Wassermann Reaction, *J. Hyg.* 39: 298, 1939.
11. Eagle, H., and Hogan, R. B.: On the Presence in Syphilitic Serum of Antibodies to Spirochetes, Their Relation to So Called Wassermann Reagin and Their Significance for the Serodiagnosis of Syphilis, *J. Exper. Med.* 71: 215, 1940.
12. Erickson, P. T., and Eagle, H.: Spirochete Complement Fixation Reaction With the Eagle and Wassermann Procedures, *Ven. Dis. Inform.* 21: 31, 1940.
13. Cappelli, E.: La "Pallidareazione" di Gachtgens sui seiri lebborose, *Gior. di batteriol. e immunol.* 22: 425, 1939.
14. Eagle, H.: Personal communication.
15. Kahn, R. L.: Are There Paradoxical Serologic Reactions in Syphilis? *Arch. Dermat. & Syph.* 39: 92, 1939.
16. Kahn, R. L.: A Serologic Verification Test in the Diagnosis of Latent Syphilis, *Arch. Dermat. & Syph.* 41: 817, 1940. See also Kahn, R. L., McDermott, E. B., and Marcus, S.: Effect of Temperature on Kahn Reaction. I. With Serologically Positive Sera of Lower Animals. II. With Serologically Positive Sera of Human Syphilis. III. With Serologically Positive Sera in the Absence of Syphilis. IV. With Serologically Negative Sera in the Absence of Syphilis, *Am. J. Syph., Gonorr. & Ven. Dis.* In press.

THE TAKATA-ARA TEST IN LIVER DISEASE*

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HISTORICAL DATA

WERE a student to seek an example in medical literature of generosity and patience on the part of investigators, he would do well to examine the case of the Takata-Ara test. He would discover in the vast number of papers on the subject, of which with comparative ease might be found more than a hundred, what has come to be the typical life history of a laboratory test in modern medicine. This includes, among other things, the obsession of devising and using a simple, and if possible, bedside test to make an absolute and never failing diagnosis in an extremely complicated and involved disease of diverse manifestations. Perhaps it is the medical equivalent of the desire to get something for nothing.

The history of the test might at once arouse suspicions. It was reported^{75, 76} at a meeting of the Far Eastern Association of Tropical Medicine for study of four widely separate diseases, no one of which may be considered as being tropical. The relative rarity of the parent publications suggests that few authors have ever consulted it. It is fair to conclude that so far as American authors are concerned, they have been content to quote from Crane that it was originally proposed by Takata "and later by Takata and Ara in 1925 as a colloid reaction in chest fluid." So far as the printed records are concerned, Takata and Ara,⁷⁵ not Takata,⁷⁶ first recorded the reaction in what may be called a prior publication, for the page is 667. Here the test was described for spinal fluid as diagnostic for syphilis and meningitis. On a later page, namely 693, and at the same meeting, Takata⁷⁶ reported on the use of the same reaction in chest fluid but in a different technical setting, to distinguish between lobar and lobular pneumonia.† So far as can be determined, study of the test has not been extended seriously in these connections. It is important to note that Takata thought the flocculation or precipitation of the mercuric salt was due to an increase in globulin, long considered the agent which causes the precipitation of various colloidal suspensions, as for example, gold sol and benzoïn.

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†It would probably be useless to attempt to obtain general agreement as to the proper name for the test if an eponym is to be used. The particular reaction resulting in a positive test may, with considerable justification, be first ascribed to Takata and Ara, since their paper preceded the paper by Takata in the volume. The serial dilution idea was also considered in the paper by Takata and Ara. However, in a limited circulated volume of abstracts of the scientific papers presented at the Sixth Congress of the Far Eastern Association of Tropical Medicine and published in Tokyo in 1925, there appeared extensive abstracts of the two papers in question. The one by Takata appeared on pages 585 to 590, while the one by Takata and Ara appeared on pages 609 and 611. The papers were evidently read at the same session and both were read by Takata, who stated that he had first used the test in the study of pneumonia. On the other hand, in neither paper was it suggested that the reaction be used as a liver function test. This was suggested by Staub,⁷² but the method of performing the test was published by Jezler.^{40, 41} It seems wise to refer to the test as the Takata-Ara test since the literature generally contains the two names linked together.

Staub⁷² knew that Adler and Strauss had shown the presence of a disturbance in the albumin-globulin ratio in the serum of persons suffering from certain liver diseases, and that Abrami and Robert-Wallich had shown it in cirrhosis in particular. He suggested to Nicole that he investigate the cause of the Takata-Ara reaction in spinal fluid, and Nicole concluded it was due to a shift in the albumin-globulin ratio. Staub then suggested to Jezler that the Takata-Ara test could be used as a liver function test on blood serum. Indeed, Staub reported that the results of the test were positive in cirrhosis, but the protocols and details were supplied by Jezler^{40, 41} the next year in his report which included a modification of the technique and a demonstration that the test could be performed on ascitic fluid. In 42 cases of cirrhosis he obtained 38 positive reactions, but he also obtained some positive reactions in pseudocirrhosis, yellow atrophy, chronic alcoholism, and passive congestion of the liver.

TABLE I

TAKATA-ARA REACTION IN CASES OF CIRRHOSIS OF THE LIVER

AUTHOR	CASES	POSITIVE REACTIONS	
		NO.	PER CENT
Jezler	176	124	70
Storz and Sehlunbaum	4	3	75
Oliva and Pescarmona	17	17	100
Skouge	40	38	95
Zadek and co-workers	6	6	100
Rohrer	15	14	93
van Ginkel	21	16	76
Crane	25	20	80
Schindel	13	12	91
Lazzaro	29	27	93
Schindel and Barth	13	12	92
Heath	77	46	60
Hafström	18	17	94
Ragins	59	57	97
Ling and Gsellmann	12	12	100
Rappolt	73	61	84
Maneke and Margaronis	66	64	97
Kirk	21	15	71
Bowman and Bray	21	18	86
Tumen and Bockus	19	15	79
Cuttle, Kerr, Wolff	18	17	94
Girard and Vincent	20	20	100
Payne	71	61	86
Heinrici	52	47	90
Horejsi	18	13	72
Israel and Reinhold	72	61	85
Wayburn and Cherry	94	87	93
Gertler and Lachenicht	21	14	67
Golob and Nussbaum	72	53	74
Gray	35	28	80
Magath	72	43	60
Total	1270	1038	82

Bauer⁵ found the test fairly satisfactory with some positive reactions in the presence of catarrhal jaundice and some negative results in cirrhosis. Storz and Sehlunbaum studied a number of cases and obtained positive reactions in 3 out of 4 cases of hepatic abscess, 3 out of 4 of cirrhosis, in 1 out of 23 of cholecystitis, and 1 out of 13 of heart failure and jaundice. Oliva and Pescarmona expressed the belief that the test is specific for cirrhosis, and this opinion has been shared by many.

For several years after this the main development of the test was along the line of demonstrating its specificity for the diagnosis of cirrhosis. In evaluating the material as a whole the fact appears that many different criteria have been used for reading a positive reaction, and that what was called a significantly positive reaction by one author is called a negative result by another. Hence the comparison of percentages in Table I must be accepted with some reservations. Furthermore, the diagnoses may be disputed in some instances, since evidence frequently was not recorded.

A careful examination of the reports, however, discloses a significant fact. In early cases of cirrhosis the Takata-Ara test frequently gives a negative result, and only in the advanced cases, usually with ascites, can it be relied on to give a positive reaction. Hence the percentage of positive reactions in a series of cases of cirrhosis may be varied by the inclusion of an excess of early or late cases. Naturally in the cases in which necropsy confirmed the presence of cirrhosis, the results of the test were almost invariably positive. In summary, the impression gained from an extensive examination of literature leads to the conclusion that in less than half of the cases of early cirrhosis a strong positive reaction will be obtained, and in a high percentage, probably 90 or 95 per cent, of the cases of late cirrhosis the results of the test will be positive.

The enthusiasm for the test, even as a diagnostic one for cirrhosis, would not have been so great if critical examination of the protocols and even the conclusions of the majority of authors had been made. It has now become apparent that parenchymatous damage of liver cells, if severe or even moderately severe, will cause a positive Takata-Ara reaction in a large percentage of cases. This damage may be caused by a wide variety of agents. It is important to know that many authors, in fact almost all who have investigated the matter, have shown that changes in the liver attributable to obstructive jaundice, even though the jaundice be severe, do not, as a rule, produce a positive Takata-Ara reaction. Crane has shown this in 18 cases; Jezler,^{40, 41} in many cases; and the same observation has been made by Storz and Schlunbaum, Wayburn and Cherry, Heath, Sommer, von Brandis, Magath, and others. Yet Gertler and Lachenicht found the test positive in all nine of their patients with "icterus simplex."

Many authors have recorded many positive reactions in the presence of parenchymatous damage of the liver which was not due to obstructive lesions. Some authors, such as Sommer, Gohr and Bolt, and Backert and Naville, have been so impressed with this that they have implied that the test may be used as an indication of such damage. Mancke and Sommer, Rathery and Ferroir, Godtfredsen, Sparchez and Viciu, Magath, Gertler and Lachenicht, Cañizo, Cornejo, and Irigoyen, and others have emphasized the frequency with which the test gives positive results in this type of hepatic injury. Such conditions so far recorded are hepatitis due to a variety of causes, abscesses, passive congestion, malignancy, hepatic syphilis, acute yellow atrophy, and possibly other conditions. Hugonot and Sohler³⁷ reported the test positive in two cases of leishmaniasis and thought the test would be positive in grave hepatic disease. Unanimity of opinion is not found among all authors, however, in regard to

this matter. For example, Crane did not find any positive reactions in 6 cases of malignancy of the liver and even thought the test could be used to advantage in identifying these cases. Von Brandis tested 30 patients with malignant disease of the liver and obtained negative results for all, yet Heinrici obtained 10 positive reactions in 12 cases. Jezler¹¹ in one series obtained positive reactions in 1 out of 6 cases studied; I found positive reactions in 7 out of 9 cases; Gertler and Lachenicht reported positive tests in 9 out of 11 cases; and Payne found 9 positive tests out of 14 cases of malignancy. Chasnoff and Solomon¹² concluded the Takata-Ara test was positive in most cases of malignancy involving the liver. Disagreement in regard to the test in passive congestion owing to cardiac failure and other conditions has also been recorded. Heinrici recorded positive reactions in half of his cases, and Horejsi, in a smaller percentage. Girard and Vincent obtained routinely negative reactions, and Storz and Schlungbaum rarely found a positive reaction. Crane recorded a few positive reactions, and Wayburn and Cherry obtained positive reactions in 7.4 per cent of their 122 cases.

In spite of many discrepancies in results, the general opinion has been that the test is useful in the diagnosis of cirrhosis. Some authors, however, have not agreed with even this general statement. Bowman and Bray stated the test was "not significant enough to be of value in the clinic as an additional laboratory procedure," and they obtained a large number of positive reactions on patients without cirrhosis whose total proteins were low or whose albumin-globulin ratios had shifted.

Many authors have recorded positive reactions in diseases not essentially hepatic in nature and even in diseases in which the liver can hardly be regarded as being essentially involved. Examples of these which may be mentioned are the positive reactions obtained in 13.4 per cent of cases of stomach ulcers, colitis, and pulmonary tuberculosis recorded by von Brandis, and in 11 out of 12 cases of pulmonary tuberculosis listed by Ornstein. Ornstein also found a high percentage of positive reactions in cases of nephritis, pellagra, secondary syphilis, chronic alcoholism, and diabetes, and even 27 per cent of positive reactions in mental maladies. Positive reactions in nephritis, pulmonary tuberculosis, arteriosclerosis and lobar pneumonia were found by Crane. Schmengler also found positive reactions in a high percentage of cases of pulmonary tuberculosis. In all cases of nephritis studied by Wayburn and Cherry positive reactions were obtained, and positive reactions also were recorded in cases of lobar pneumonia and pulmonary tuberculosis. Biondo found positive reactions in two cases of pulmonary tuberculosis and atrophic changes in the liver. Girard and Vincent emphasized the likelihood of positive reactions in the presence of nephritis with low serum proteins, and others have recorded occasionally positive reactions in a variety of diseases. Weiner also obtained positive reactions in nephritis. Banti's disease has not given consistent reactions in the hands of various authors; some recorded positive reactions and others negative ones.

Hirsch, after observing the test in a long series of children, concluded that the test was more than a liver function test; that its results were positive in all serious diseases in which the detoxicating function of the liver was disturbed or

the colloidal state of the serum was changed. This among children occurs frequently in such diseases as typhoid fever, pneumonia, nephritis, and in septic conditions.

It is interesting to note that Bauer,⁶ and more recently Gray, have reported on colloidal gold tests on serum of patients with hepatic disease. They have found in general that positive reactions are the rule in cases of cirrhosis and acute parenchymatous hepatic disease, and occur in many cases of malignancy of the liver and various hepatic diseases. This colloidal gold test was found to give a positive reaction in certain extrahepatic diseases and in particular in syphilis. The test appears to be more sensitive than the Takata-Ara test as a general liver function test and hence less specific for cirrhosis. Gray thought the test did not depend primarily upon a quantitative increase in globulin, a decrease in albumin, or an inversion of the albumin-globulin ratio. He was of the opinion that euglobulin was the key to the reaction.

METHOD

The technique described by Takata⁷⁵ required eleven tubes. One cubic centimeter of patient's serum was so diluted with physiologic salt solution that a serial dilution resulted as follows: 1:5, 1:10, 1:20, to 1:500. To each tube were added 0.25 c.c. of 10 per cent solution of sodium carbonate and 0.25 c.c. of a fresh mixture of mercuric chloride solution, consisting of equal parts of 0.5 per cent sublimate solution and 0.02 per cent fuchsin. The tubes were allowed to stand for several hours, and positive results were indicated by flocculation in the tubes. Jezler⁴¹ modified the test by using nine tubes and making a serial dilution beginning with 1 c.c. of serum and 1 c.c. of salt, in the proportion of 1:2, then 1:4, 1:8, to 1:512. To these dilutions were added 0.25 c.c. of 10 per cent solution of sodium carbonate and 0.3 c.c. of sublimate fuchsin mixture. The reading was made after a half hour and again after five hours, and positive reactions were indicated when at least three tubes of 1:32 dilution or higher showed flocculation. Takata and Ara used a single tube into which were placed 1 c.c. of spinal fluid, 1 drop of 10 per cent solution of sodium carbonate, and 0.3 c.c. of a mixture of 0.5 per cent solution of mercuric chloride and 0.02 per cent solution of fuchsin. The tube was shaken and allowed to stand for twenty-four hours. They also proposed the serial dilution scheme.

Since Takata and Ara announced their test a large number of modifications have been introduced. One which has been used extensively and which was used in my previously reported series was a modification proposed by Heath. For this test six tubes are used, and into each is placed 1 c.c. of 0.9 per cent solution of sodium carbonate. To the first tube 1.0 c.c. of serum is added, and the contents are thoroughly mixed. One cubic centimeter then is removed and added to the second tube. After mixing, 1.0 c.c. is transferred from the second to the third tube, and so on until the sixth tube, from which 1.0 c.c. is discarded. The dilutions of serum thus range from 1:2 to 1:64. To each of the tubes 0.25 c.c. of 10 per cent solution of sodium carbonate is added, and the contents are well shaken. Then 0.15 c.c. of solution of mercuric chloride is added to each tube, and the tubes are again shaken. The mixtures are allowed to stand at room temperature overnight, and the readings are made at sixteen to twenty-four hours. Heath

divided the reactions into five groups: strongly positive, positive, weakly positive, suspicious, and negative. A strongly positive reaction is characterized by a maximal precipitate on settling, filling at least the lower third of the fluid column. A positive reaction occurs when there is a definite but not a maximal precipitate in any one tube, or some precipitate in at least three tubes. A weakly positive reaction is present when there is a definite but minimal precipitate in any one or two tubes, and a suspicious reaction is present when there is a doubtful precipitate in any two tubes. The reaction is considered negative when there is no flocculent precipitate. A definite, pearly flocculent precipitate must be present to interpret a reaction as positive. Granular or flaky precipitates are disregarded.

Ueko's^{80, 81} modification consisted of using four tubes, into each of which is placed 0.2 c.c. of serum; and into the first tube, 0.25 c.c. of 0.36 per cent solution of sodium carbonate; into the second tube, 0.2 c.c.; into the third, 1.5 c.c., and into the fourth, 1 c.c. Solution of mercuric chloride, 0.5 per cent, is added to each tube in amounts equal to the carbonate solution. If three or more tubes are translucent after they have stood for several hours, the reaction is said to be negative. If there is an opaqueness with some precipitate in the first three tubes, it is graded 1+. If there is a precipitate or flocculation of uniform turbidity in the four tubes, it is graded 2+; and if a thick precipitate forms immediately in all four tubes, the reaction is graded 3+. Ueko⁸¹ thought his modification gave a more certain indication of disturbance of the liver than did the Takata test, and on comparison obtained about 20 per cent disagreement between the two tests. Bauer⁵ used magnesium chloride instead of mercuric chloride.

Many suggestions for reading the results of the test have been proposed, as for example, the one by Hafström, who graded the results on a scale of 1 to 4: grade 1 being slight turbidity; grade 2, incipient flocculation; grade 3, clear flocculation; and grade 4, maximal flocculation. Hafström also used fuchsin in his tests.

Mancke and Sommer devised a modification which they advocated: Into each of eight tubes is placed 0.1 c.c. of the serum; then 1.0 c.c. of the salt solution (0.9 per cent) is put into the first tube, 1.1 c.c. in the next, and so on, until the last tube receives 1.7 c.c. Into each tube is placed 0.4 c.c. of a 10 per cent solution of sodium carbonate, and then 1.0 c.c. of 0.25 per cent solution of mercuric chloride is pipetted into the first tube and 0.9 c.c. in the second; the quantity is decreased by 0.1 c.c. until the last tube receives 0.3 c.c. The flocculation test is read after twenty-four hours.

Since Wayburn and Cherry indicated that their method of performing the test increased its accuracy, and since they did not altogether agree with my previous report, I decided in this new series to use their technique.* The method of Wayburn and Cherry is a modification of that proposed by Crane and is not unlike the method suggested by Heath: eight Wassermann tubes are used

*Both in the tables and text of Wayburn and Cherry's paper they indicated that I found 9 positive Takata-Ara reactions in 25 cases of cirrhosis of the liver, and in the table they indicated that the percentage of positive reactions was 36 per cent, which would have brought my results entirely out of line with all previous results. Evidently this is a misreading on the part of Wayburn and Cherry. In 16 or 64 per cent of my series of 25 cases of cirrhosis of the liver, the Takata-Ara reaction was positive. This may be compared favorably with Heath's 60 per cent and Kirk's 71 per cent, as reported in the table by Wayburn and Cherry.

instead of six, and the serum is serially pipetted in geometric dilutions. The tubes are agitated thoroughly for thirty to forty seconds after receiving 0.25 c.c. of 10 per cent solution of sodium carbonate, and the individual tubes are shaken as soon as 0.15 c.c. of a 0.5 per cent solution of mercuric chloride is added. The tubes are left at room temperature for twenty-four hours before reading. The presence of a characteristic precipitate is considered a positive reaction. This is a white to pearl gray, soft, large-flaked flocculent. The serum was not considered positive unless a precipitate appeared in at least three consecutive tubes. This usually begins in the 1:8 dilution. The appearance of a fine, brick-red precipitation is of no significance. Wayburn and Cherry admitted that the six tubes proposed by Crane were rational, although they thought the results are demonstrated more clearly with eight tubes. I did not notice the advantage of the two additional tubes.

Although the technique of performing the Takata-Ara test is superficially simple, careful study and experience in reading precipitation tests are necessary in order to secure satisfactory results. Frequently precipitation occurs that evidently is not colloidal flocculation and gives the inexperienced worker difficulty in interpreting the test.

MECHANISM OF THE TAKATA-ARA TEST

In spite of the vast literature which has accumulated concerning the mechanism of the Takata-Ara test, there is as yet no general agreement or adequate explanation for the flocculation that occurs in the serum of certain patients. In general, it is believed that mercuric chloride and sodium carbonate form mercuric oxide in the presence of proteins which are considered to act as protective colloids. In certain pathologic states precipitation of the colloid occurs because as is generally believed, the albumin-globulin ratio in the serum has been altered. Takata⁷⁰ thought that the ratio was altered by an increase in the globulin fraction. Jezler^{40, 41} found the globulin fraction regularly more than 55 per cent of the total protein in cases in which Takata-Ara reactions were positive; whereas in cases in which the results were negative the average was about 37 per cent.

Gros,²⁹ from a comprehensive study of the mechanism of the Takata-Ara reaction, concluded that mercuric chloride was adsorbed in organic substances onto the protein, but he did not think that this was specific for persons with diseases of the liver.

Many investigators have concluded that the precipitation by the alkaline sublimate solution was due to a disturbance in the albumin-globulin ratio, although many have noted that in a fairly large number of cases a positive Takata-Ara reaction has been obtained when the ratio was normal, and conversely, negative reactions have been seen when the ratio was disturbed. Some authors have stated that a mere lowering of the protein level did not result in flocculation, although Abrami and Robert-Wallich thought it did. Among those who have held that a change in the ratio between albumin and globulin resulting in a relative increase in globulin was the cause of the precipitation may be mentioned Lazzaro, Vigada and Montanari, Hugonot and Sohler,³⁶ Schreuder, Carrière, Martin, and Dufossé, Ragins, and Gohr and Niedeggen. Others, such as van

Ginkel, Gros,²⁸ Hahn, Rappolt, Kirk, and Ueko,³² indicated that an actual increase in the serum globulin must be considered important in causing the positive reaction.

In opposition to these opinions, Skouge, and Schindel and Barth were unable to correlate accurately the Takata-Ara reaction with either an increase in globulin or a lowering of the albumin-globulin ratio. Fulde thought there was no correlation between the albumin-globulin shift and the reaction. Schreuder took the half way position, that either an increase in globulin or a decrease in albumin would cause a positive reaction. When Kallos-Deffner starved rabbits in a rarefied atmosphere and produced a ketonuria in them, the Takata-Ara test then gave a positive result which became negative when the animals returned to normal; d'Alessandro corroborated these observations. Recht lent some support to this line of thought when he demonstrated the positive reaction of children with acidosis and dehydration.

Medvei and Paschkis, Zirm, and Ragins have noted that after the addition of heparin to blood, the serum which previously had given a positive Takata-Ara reaction gave a negative reaction; this occurred in spite of the fact that Fischer demonstrated heparin converts albumin in serum into globulin.

Bowman and Bray obtained many positive Takata-Ara reactions with serum in which the level of protein was low and in which the albumin-globulin ratio was not reversed.

Tumen and Bockus did not think that the reaction was wholly dependent on the albumin-globulin ratio, although they stated that a low albumin content and the reversal of the albumin-globulin ratio were the rule. The same conclusion was reached by Cuttle, Kerr, and Wolff. Chasnoff and Solomon,¹⁵ after studying 28 cases, concluded that in most cases there was an alteration in the albumin-globulin ratio when the Takata-Ara reaction was positive; although this was the most important cause of the reaction, it was not the sole cause.

Taran and Lipstein pointed out that the albumin levels are lower and the globulin levels are higher in serum with a positive reaction. In their series of 40 cases of amyloidosis and tuberculosis, Takata-Ara reactions were positive in 31. These authors, however, expressed the belief that this was not due to the presence of amyloid but to the changes in the relation of the serum proteins.

Schindel produced positive reactions by the addition of lower fatty acids to serum and reversed the reaction when he then added alkalis, yet Gros²⁸ could produce no changes in the test after extraction of fatty acids from the serum. Ueko³⁰ rightly criticized these experiments as being invalid, since the addition of fatty acids changes the reaction in the tubes to such an extent that the test is no longer comparable to the original test.

Oselein found the ammonia content of the blood high in cases in which the Takata-Ara reactions were positive. When the amount of ammonia was low, that is, from 0.2 to 0.58 mg. per 100 c.c., he found the reaction was negative. It was positive when the quantity of ammonia was above the level of 0.85 mg. per 100 c.c. Chasnoff and Solomon,¹⁵ after an exhaustive study of this factor, concluded the ammonia content of the blood bore no relation to the Takata-Ara test.

Horejsi contended that the globulin in the serum caused the precipitation in the Takata-Ara reaction when the globulin was not protected. He suggested

this protection was due to "some sulphuric compound in the blood serum," perhaps a free SH-group. He explained the reaction in hepatic disease on the theory that the sulfur metabolism was faulty in such diseases.

Seiler and Rehm maintained that this reaction was not a colloid-colloid reaction, but that the mercuric chloride in the Takata-Ara test was in a true solution, entering into the protein complex and remaining as a sublimate or oxychloride. They were of the opinion that the flocculation was primarily a pH reaction present in pathologic sera.

Staub and Jezler concluded the Takata-Ara reaction was a result of dysfunction of the liver, dependent on the colloidal composition of the serum. Jürgens, from experiments on geese and dogs, concluded that the test was dependent on metabolic products of damaged liver cells, and Crane suggested as possible causes of a positive reaction: (1) release of liver proteins into the circulation (that is, in cases of yellow atrophy); (2) inability of the liver to alter proteins contained in the blood; and (3) failure of some blood to pass through the liver, going instead, by way of collateral venous anastomoses, directly into the general circulation.

Another suggestion has been made by Reeht, that lipoids played an important role in the test.

Wuhrmann and Leuthardt held that the precipitate was due to the linkage of sublimate with proteins of the blood serum in which fibrinogen also played a part; others have suggested that fibrinogen played some role in the reaction.

One of the few papers based on animal experimentation on the subject is by Hahn, who made mixtures of calf and beef blood so as to obtain different albumin-globulin ratios. He concluded that as the ratio of globulin to albumin increased, results of the Takata-Ara test became more positive. Wayburn and Cherry have summarized their views as follows:

"It is extremely difficult to explain the occurrence of the positive Takata-Ara reaction by any one hypothesis. Increase in the globulin fraction of the serum, both relatively and absolutely, does occur in the majority of the cases, but there are many cases in which it does not. Conversely, there are many cases with changes in the globulin without positive Takata reactions. The presence of liver damage as such appears to play very little role. If the release of liver proteins into the circulation were important, acute yellow atrophy should give uniformly strongly positive reactions, but it does not. If the reaction were caused by the failure of blood to pass through the liver, portal thrombosis should give uniformly positive reactions, but it does not. It seems rational at this time to be content with Staub and Jezler that the Takata reaction represents a dysfunction of the liver. It probably is dependent on the colloidal composition of the serum. It probably represents some retention substance which under the usual conditions of its occurrences requires some time to be formed in sufficient quantity to result in a positive reaction (comparable, again, to an elevated blood urea in chronic glomerular nephritis). It is possible that this is a toxic product."

The main objection to this summary is their agreement with Staub and Jezler and the suggestion that the reaction is due to a toxic or retention substance. Greene, and others as well, have pointed out that the results of the test

are often positive when the liver is not damaged to the extent that any other tests or observations can demonstrate such damage and also are positive in diseases in which there is no indication the liver is involved.

The reaction is likely to be due to a more fundamental change in the body fluids, which often, perhaps more often, is due to dysfunction of the liver than to that of any other organ but which may be caused by the dysfunction of other organs, and results in colloidal changes in various bodily fluids, such as serum, spinal fluid, and the fluids secreted by serosal surfaces.

RESULTS OF OBSERVATIONS

In order to investigate the value of the Takata-Ara reaction, the two series of cases which I have studied have been combined, since a separate examination of each series did not reveal any particular differences. The results in the first series have been reported previously, and this series consisted of 86 consecutive cases. In all but one instance the bromsulfalein test was performed simultaneously, and in all but three, qualitative and quantitative van den Bergh reactions were studied. Furthermore, examinations of the serum protein were made in 62 of these cases. All these patients were carefully examined by experienced clinicians, with special reference to the possibility of disease of the liver. The condition of the liver of 27 of these was observed either at operation or at necropsy. The method of performing the test was the six-tube method suggested by Heath.

Since study of the second series was stimulated by the criticism of Wayburn and Cherry, their technique of using eight tubes was followed. The cases in this series were selected in an entirely different manner from that used for selection in the first series. Only cases were included in which the bromsulfalein test indicated retention of 6 per cent or more (grade 1) of dye; the Takata-Ara test was performed on the same sera. All these patients had both qualitative and quantitative van den Bergh reactions. In this series the livers of 50 patients were observed either at operation or at necropsy, and all the patients in the series, like those of the former series, were studied carefully by clinicians, with particular reference to the possibility of disease of the liver.

The combined series included 249 patients. The condition of 72 of these patients was diagnosed as cirrhosis of the liver; of this number, 43, or about 60 per cent, had a positive Takata-Ara reaction (Table II). Thirty had malignant disease involving the liver; of these, 12, or 40 per cent, gave positive Takata-Ara reactions. The livers of 108 patients were involved in other types of disease, of which 20, or 19 per cent, gave positive Takata-Ara reactions. In all, there were 210 cases of clear-cut involvement of the liver, in 75, or 40 per cent, of which Takata-Ara tests gave positive results. When the cases of cirrhosis of the liver were omitted, positive reactions were found in 23 per cent of the other cases in which the liver was involved. Eighteen patients in the second series had more or less acute hepatitis, and 5, or 28 per cent, of these gave a positive Takata-Ara reaction. In this series, which obviously was selected in favor of involvement of the liver, positive Takata-Ara reactions were noted in 5 instances in which there evidently was no involvement of the liver; results of

the Takata-Ara test were negative in 12 cases in which obviously the liver was not injured. In this series, as in some reported by others, obstructive lesions did not result in positive reactions, and in all kinds of early lesions of the liver the results of the tests were usually negative.

TABLE II

TAKATA-ARA TEST AS RELATED TO LIVER DAMAGE. FIRST AND SECOND SERIES COMBINED (249 CASES)

CONDITION OF LIVER*	TAKATA-ARA TEST			
	POSITIVE		NEGATIVE	
	CASES	PER CENT	CASES	PER CENT
Cirrhosis present	43	60	29	40
Cirrhosis absent	41	23	136	77
Malignancy of liver	12	40	18	60
Liver involved†	20	19	88	81
Liver not involved	3	23	30	77

*There were 210 cases in which the liver was involved; in 75, or 36 per cent, the results of the Takata-Ara test were positive. When the cases of cirrhosis are omitted, the Takata-Ara test was positive in 23 per cent.

†Other than diseases listed above.

TABLE III

TAKATA-ARA TEST OF LIVER FUNCTION IN FIRST SERIES (86 CASES)

RESULTS OF VARIOUS TESTS	TAKATA-ARA TEST	
	POSITIVE	NEGATIVE
Albumin-globulin ratio, reversed	12	2
Albumin-globulin ratio, normal	9	39
Bromsulfalein test, positive	28	29
Bromsulfalein test, negative	4*	24
van den Bergh reaction, direct	22	19
van den Bergh reaction, indirect	9†	33

*Liver apparently not involved.

†In 4 of these cases the liver apparently was not involved.

There was no evidence to suggest that changes in the total protein of the serum could be correlated with the Takata-Ara tests; this was studied in the first series in particular (Table III). In 12 cases in which results of the Takata-Ara test were positive, there was a reversal of the albumin-globulin ratio, and in 9 there was a normal ratio; in 2 cases the ratio was reversed, but results of the Takata-Ara test were negative. In only about one-half of the cases in which there was a reversal of the albumin-globulin ratio was the liver involved, and in 9 out of 21 cases of cirrhosis the ratios were reversed (Table IV). In only one case, that of hypernephroma, the ratio was reversed, and involvement of the liver was not diagnosed. It should be noted, however, that very few cases of disease of the kidney were included in this series. It is possible that the fibrinogen content of the serum has an influence on the Takata-Ara reaction and may account for Takata's⁷⁵ reaction in cases of pneumonia.

The correlation between the Takata-Ara test and the bromsulfalein test was not high. In only half of the cases of the first series in which retention of dye occurred were the results of the Takata-Ara tests positive. Since the second series included only cases in which retention of dye of some grade was present with the exception of a single case, the correlation can be examined only from one side. Of 163 cases 51 gave a positive Takata-Ara reaction.

TABLE IV

ALBUMIN-GLOBULIN RATIO IN DISEASE OF LIVER IN FIRST SERIES

CONDITION OF LIVER	ALBUMIN-GLOBULIN RATIO	
	NORMAL CASES	REVERSED CASES
Cirrhosis present	12	9
Cirrhosis absent	36	4*
Cells of liver involved	23	13
Cells of liver not involved	25	1†

*Carcinoma of liver present in 3 of these cases, hypernephroma in the fourth.

†Hypernephroma.

The van den Bergh reaction was indirect in a large number of instances in which the Takata-Ara reaction was positive. In the present series direct van den Bergh reactions were present when results of 27 Takata-Ara tests were positive and when 23 were negative.

Ten patients in the present series had syphilis, 7 of whom gave positive results to the Takata-Ara test. The liver was involved in all of these patients.

TABLE V

COMPARISON OF TESTS OF LIVER FUNCTION IN FIRST SERIES

TEST AND RESULT	CELLS OF LIVER	
	INVOLVED CASES	NOT INVOLVED CASES
Takata-Ara test, positive	28	4*
Takata-Ara test, negative	25	29
Bromsulfalein test, positive	51	3†
Bromsulfalein test, negative	2‡	29
van den Bergh reaction, direct	39	2§
van den Bergh reaction, indirect	13	29

*Cases of myocardial degeneration, adenoma of gall bladder, hemolytic icterus, and polythemia, respectively.

†Cases of arthritis, hypernephroma, and facial neuralgia, respectively.

‡Hepatitis (?) and syphilis present in one case; recession in cholangitis in the other.

§Hypernephroma in one case, hemolytic icterus in the other.

As was pointed out in a previous paper, the results of the bromsulfalein test were extremely significant. In the first series, results of the bromsulfalein test were positive in 51 cases in which the liver was involved by disease, and in only 3 cases in which the liver was thought to be uninvolved (Table V). Even in these 3 cases, however, there may have been some involvement of the liver. In the present series there were 12 cases in which no clinical diagnosis of involvement of the liver was made, but it is interesting to note that in 5 of these the diagnosis of gout was recorded. Since an increase in uric acid in the blood is present in gout and since Bollman, Mann, and I have shown that the liver is involved in the metabolism of uric acid, the livers of these patients may not have been normal and the retention of dye may have been valid. In 2 cases there were malignant lesions of other organs.

Again the importance of retention of dye of low grade, up to 12 per cent retention, is brought out, since in a large number of cases of involvement of the liver retention of dye grade 1 was present (Table VI). There were in the present series 9 cases of Banti's disease, and in 6 of these Takata-Ara reactions were positive. This is in contrast to the experience of Wayburn and Cherry, who stated that in their series of 3 cases of Banti's syndrome and 2 of splenic

and portal thrombosis the results of the Takata-Ara test were consistently negative. In one case of multiple myelomas the Takata-Ara reaction was positive but there was no retention of dye.

TABLE VI

TAKATA-ARA TEST, BROMSULFALEIN TEST AND VAN DEN BERGH REACTION IN PRESENT SERIES

CONDITION	CASES	BROMSULFALEIN TEST GRADE				VAN DEN BERGH REACTION			
						DIRECT	INDIRECT	LESS THAN 2 MG. PER 100 C.C.	MORE THAN 2 MG. PER 100 C.C.
		1	2	3	4				
TAKATA-ARA TEST NEGATIVE									
Hepatitis	13	4	6	3	0	8	5	10	3
Malignant lesion of liver	16	3	3	9	1	5	11	14	2
Cirrhosis	20	9	7	1	3	3	17	16	4
Other diseases	62*	37	20	5	0	7	55	58	4
TAKATA-ARA TEST POSITIVE									
Hepatitis	5	0	1	2	2	5	0	1	4
Malignant lesion of liver	5	2	3	0	0	1	3	3	2
Cirrhosis	27	5	6	13	3	17	10	16	11
Other diseases	15†	1	5	7	1	4	12	14	1

*In 12 of these cases no clinical diagnosis of involvement of the liver was made, but in 5 of them a diagnosis of gout was made.

†In one of these no clinical diagnosis of involvement of the liver was made and the bromsulfalein test gave negative results; in 4 other cases also no involvement of the liver was found.

COMMENT

From an examination of the literature, as well as from my own series of cases, it becomes evident that the Takata-Ara reaction has not been explained on any absolute basis.

It is, therefore, an empiric test. A wide variety of explanations have been offered to account for the reaction, and different explanations of its chemical or physical mechanism have been defended. The evidence supports the conclusion that in a high percentage of positive reactions the albumin-globulin ratio is reversed and that in even a higher percentage there is some alteration in either the relative or the absolute amounts of these proteins. On the other hand, the role of fibrinogen, as well as that of other contents of the serum, has not been investigated thoroughly. Important are the observations that in an appreciable number of cases the albumin-globulin ratio is not disturbed when the Takata-Ara reaction is positive; this indicates that some other factor or factors must be present to explain the phenomenon.

It is more than evident that the test is not specific for cirrhosis of the liver. For the conditions the test was first proposed to diagnose there is no reason to believe that it has any value. It is evident enough that a large number of patients with cirrhosis will have positive reactions, and the more extensive the cirrhosis and the longer the disease has lasted, the more likelihood of obtaining a positive reaction. The degree of positiveness of the test is not indicative of the degree of hepatic injury, and it is evidently clear that fine laboratory subdivisions of readings of the test will only confuse the clinician. In spite of the

fact that a positive result of the test may be expected in 82 per cent of cases of cirrhosis, it cannot be assumed that given a positive reaction such a diagnosis is confirmed. This, of course, is because a positive reaction occurs in so many other conditions. Jankelson, Segal, and Aisner reported that the Takata-Ara test gave about the same percentage of positive results, namely 80, in cirrhosis as did their tyrosine test. If it were desired, however, to consider the test as a general test of liver function, the results would be disappointing. It certainly will not compare favorably with either the bromsulfalein test which, when properly performed,* will indicate roughly 95 per cent of cases with hepatic injury, or the van den Bergh test. If a direct van den Bergh reaction is used as a criterion of injury to the liver, it will be found to be reliable in about 80 per cent of cases. One may expect only about 1 per cent of cases to yield a direct reaction in the absence of clinical evidence of hepatic damage if careful ring tests are performed. Indirect van den Bergh reactions will occur until the damage to the liver is great enough to render the organ unable to excrete the changed bilirubin through the bile passages.

The question may be reasonably asked if the Takata-Ara test can be used to denote intrahepatic injury as distinguished from extrahepatic pathologic changes, with subsequent effects on the liver, or at least on the biliary system. A fair appraisal would suggest that the test is about as valuable as some of the proposed tests for this purpose. Unless obstruction has been present for a long time, a negative result will be obtained. Unfortunately, unless the parenchymal damage is severe or moderately severe, the test also gives negative results, and this means the test is least useful when most desired. Judiciously used as a differential test between acute hepatitis with jaundice and obstruction of the bile ducts, the test should prove of value, although not entirely accurate.

Investigators must not lose sight of the fact that the Takata-Ara test may be positive and often is in many kinds of diseases in which the liver may not be involved, as for example, syphilis, nephritis, pneumonia, and pulmonary tuberculosis. If there is a substance in, or a condition of, the serum common to the wide variety of diseases which yield positive results to the test, its nature has not been elucidated.

It is unlikely the test would ever have enjoyed such widespread discussion if the technique had not appeared to be easy. Indeed, most authors have praised the simplicity of the test, as if that made up for its shortcomings. If the test is so simple, why have so many modifications arisen in fifteen years and such divergent results been obtained by different authors? Bots listed fourteen modifications and, incidentally, recommended the one of Jezler,⁴² who wrote that it was not so much a matter of the particular method used but the interpretation of the test. The truth of the matter is that the test, which is empiric, of unknown mechanism and colloidal in nature, is not simple either to perform or to read. The strictest attention to details is necessary, and even faulty preparation of glassware can be a frequent source of error. The reading of such a test as the Takata-Ara requires experience in a wide variety of similar procedures and should not be attempted by a novice. Even after the correct reading is

*The dose should be 5 mg. per kilogram of body weight, and the lowest grade of retention of dye to be considered positive should be 6 per cent at the end of one hour.

made, its interpretation is still a matter for the most experienced, for after fifteen years there is no agreement as to the degree of flocculation necessary or the number of tubes involved to constitute a positive result. No wonder its true value has not been established.

Some investigators have maintained the test was of value in prognosis, recording correlated shifts in the test as patients improved or became worse. The fact that the test often gives negative results in early cirrhosis and positive results in late cases at once indicates the rationale of such a suggestion. There is not enough information about the matter as yet to form any definite conclusion about the prognostic value of the test, but from my own experience I would expect it to follow changes in the condition of the patient rather than to precede such changes, so that although it would in a general way parallel the patient's condition, it could not be used often to predict the course of events in any given case. Gertler and Lachenicht contended that a positive test was an important prognostic sign, and once a patient has a positive test he does not often completely recover from his malady. It is doubtful whether it will surpass the van den Bergh as a prognostic test.

Much evidence supports the view that the Takata-Ara test is not very sensitive. Hence, there is no need to perform the test if the bromsulfalein test gives negative results. If results of the dye test are positive and a Takata-Ara test is then performed, a positive reaction will usually indicate a severe grade of parenchymatous damage, and this may help in the diagnosis. If the result of the test under this circumstance is negative, the chance that the patient has cirrhosis is lessened and here it may render a definite service.

SUMMARY AND CONCLUSIONS

The Takata-Ara test is an empiric one and may give positive results in a wide variety of diseases, in particular, parenchymatous diseases of the liver, pulmonary tuberculosis, and nephritis. It is not very sensitive.

It is not a specific test for any one disease, but it usually gives positive results in cirrhosis of the liver, especially when the disease has progressed to moderate severity. The reaction is frequently positive in acute hepatitis and in malignant disease of the liver.

The test is correlated to a great extent with changes in the ratio of serum albumin to globulin, but the correlation is not absolute and the mechanism and cause of the reaction remain unexplained.

It has some slight value in prognosis, but its true significance in this field has not been established. It appears to have greater value in establishing the general nature of an hepatic injury—whether it is of extrahepatic or intra-hepatic origin—although more information in this regard is needed.

The many modifications of the technique of the test and different methods of interpreting its results not only make the performance of it confusing, but also cast great doubt on the oft-repeated statement that the test is a simple one.

If the test is to survive there is great need for the establishment of a standard procedure based on a long and varied experience with the test. A careful reading of the literature, however, does not suggest that it would be worth the effort.

REFERENCES

1. Abrami, P., and Robert-Wallich: Modifications du sérum sanguin au cours des cirrhoses du foie avec ascite; inversion du rapport sériens-globulines, *Compt. rend. Soc. de biol.* 101: 291, 1929.
2. Adler, Erich, and Strauss, Leo: Beitrag zum Mechanismus der Bilirubinreaktion im Blutserum. Experimentell-klinische Untersuchungen. II. Mitteilung, *Ztschr. f. d. ges. exper. Med.* 44: 9, 1924.
3. d'Alessandro, R.: Contributo al meccanismo della reazione di Takata-Ara; ricerche sperimentali, *Policlinico (sez. med.)* 42: 612, 1935.
4. Backert, Paul: Die Takata-Reaktion nach Ucko in ihrer Bedeutung für den praktischen Arzt, *Zentralbl. f. inn. Med.* 59: 689, 1938.
5. Bauer, Richard: Unsere Kenntnisse über Leberfunktion und ihre Verwertung für die Klinik, *Wien. klin. Wchnschr.* 45: 1577, 1932.
6. Bauer, Richard: Eino novo Seroreaktion—Serum-Goldsolreaktion, *Klin. Wchnschr.* 16: 1570, 1937.
7. Biondo, Antonino: Sul valore diagnostico e prognostico della reazione di Takata Ara nel siero di sangue dei malati di fegato, *Policlinico (sez. prat.)* 41: 1681, 1934.
8. Bollman, J. L., Maun, F. C., and Magath, T. B.: Studies on the Physiology of the Liver. X. Uric Acid Following Total Removal of the Liver, *Am. J. Physiol.* 72: 629, 1925.
9. Bots, P.: Über neue Modifikationen der Takataschen Loberfunktionsprobe, *Schweiz. med. Wchnschr.* 68: 1230, 1938.
10. Bowman, R. O., and Bray, R. S.: An Evaluation of the Takata-Ara Reaction for Diagnosis of Liver Cirrhosis, *J. LAB. & CLIN. MED.* 22: 532, 1937.
11. von Brandis, H.-J.: Leberfunktionsprüfung und chirurgische Anzeigestellung unter besonderer Berücksichtigung der Reaktion nach Takata-Ara und des Cholesterinestersturztes, *Beitr. z. klin. Chir.* 168: 565, 1938.
12. del Cañizo, J., Cornejo, A., and Irigoyen, A.: La reacción de Takata en las enfermedades hepáticas, *Rev. españ. de enferm. d. ap. digest. y de la nutrición* 2: 403, 1936.
13. Carrière, G., Martin, P., and Dufossé, A.: La valeur de la réaction de Takata Ara pratiquée sur le sérum, *Gaz. d. hôp.* 108: 1041, 1935.
14. Chasnow, Julius, and Solomon, Sydaey: The Takata-Ara Reaction. I. Clinical Significance, *J. LAB. & CLIN. MED.* 23: 887, 1938.
15. Chasnow, Julius, and Solomon, Sydney: The Takata-Ara Reaction. II. Mechanism—With Special Reference to the Influence of Ammonia Blood Level, *J. LAB. & CLIN. MED.* 23: 894, 1938.
16. Crane, M. P.: A Modified Mercuric Chlorid Reaction (Takata-Ara) in Cirrhosis and in Neoplasms of the Liver, *Am. J. M. Soc.* 187: 705, 1934.
17. Cottle, T. D., Kerr, E. G., and Wolff, W. A.: Observations on the Takata-Ara Test in Hepatic Diseases, *Bull. Ayer Clin. Lab., Pennsylvania Hosp.* 3: 99, 1937.
18. Fulde, Walter: Wert und Methodik verschiedener Leberfunktionsprüfungen für Klinik und Praxis, *Klin. Wchnschr.* 14: 1201, 1935.
19. Gertler, H., and Lachenicht, P.: Über die Takata-reaktion; Beobachtungsergebnisse bei Krankheiten der Leber und Gallenwege, *Ztschr. f. klin. Med.* 132: 144, 1937.
20. van Ginkel, J. H. R.: Takata-Ara Test in Cirrhosis of the Liver, *Nederl. tijdschr. v. geneesk.* 78: 991, 1934.
21. Girard, M., and Vincent, D.: Intérêt de la réaction de flocculation de Takata-Ara dans le diagnostic des maladies du foie, *Lyon méd.* 160: 672, 1937.
22. Godfredsen, Erik: Om værdien af Takata-reaktionen ved leverlidelse, *Nord. med. (Hospitalstid.)* 5: 131, 1940.
23. Gohr, H., and Bolt, W.: Vergleich der Serumreaktionen nach Bauer, Takata und Weltrich unter Berücksichtigung des Albumin/Globulinquotienten und des . . . f. klin. Med. 137: 29, 1939.
24. Gohr, H.: . . . Erfahrungen mit der Takata-Ara-Reaktion ihre Beziehung zu . . . s Serums und zum Albumin-Globulinquotienten, *Klin. . .*
25. Golob, M., and Nussbaum, C.: Evaluation of Takata-Ara Reaction as Liver Function Test, *Am. J. Digest. Dis.* 6: 200, 1939.
26. Gray, S. J.: The Colloidal Gold Reaction of Blood Serum in Diseases of the Liver, *Arch. Int. Med.* 65: 524, 1940.
27. Greene, C. H.: Liver and Biliary Tract; A Review of Certain Recent Contributions, *Arch. Int. Med.* 57: 1039, 1936.
28. Gros, W.: Ueber die Bedeutung der normalerweise im Serum vorkommenden niederen Fettsäuren für die Takata'sche Reaktion, *München. med. Wchnschr.* 2: 1151, 1935.
29. Gros, Walther: Das Bluteiweißbild und seine Bedeutung für den Mechanismus der Takata-Reaktion, *Ztschr. f. d. ges. exper. Med.* 101: 519, 1937.

made, its interpretation is still a matter for the most experienced, for after fifteen years there is no agreement as to the degree of flocculation necessary or the number of tubes involved to constitute a positive result. No wonder its true value has not been established.

Some investigators have maintained the test was of value in prognosis, recording correlated shifts in the test as patients improved or became worse. The fact that the test often gives negative results in early cirrhosis and positive results in late cases at once indicates the rationale of such a suggestion. There is not enough information about the matter as yet to form any definite conclusion about the prognostic value of the test, but from my own experience I would expect it to follow changes in the condition of the patient rather than to precede such changes, so that although it would in a general way parallel the patient's condition, it could not be used often to predict the course of events in any given case. Gertler and Laehenicht contended that a positive test was an important prognostic sign, and once a patient has a positive test he does not often completely recover from his malady. It is doubtful whether it will surpass the van den Bergh as a prognostic test.

Much evidence supports the view that the Takata-Ara test is not very sensitive. Hence, there is no need to perform the test if the bromsulfalein test gives negative results. If results of the dye test are positive and a Takata-Ara test is then performed, a positive reaction will usually indicate a severe grade of parenchymatous damage, and this may help in the diagnosis. If the result of the test under this circumstance is negative, the chance that the patient has cirrhosis is lessened and here it may render a definite service.

SUMMARY AND CONCLUSIONS

The Takata-Ara test is an empiric one and may give positive results in a wide variety of diseases, in particular, parenchymatous diseases of the liver, pulmonary tuberculosis, and nephritis. It is not very sensitive.

It is not a specific test for any one disease, but it usually gives positive results in cirrhosis of the liver, especially when the disease has progressed to moderate severity. The reaction is frequently positive in acute hepatitis and in malignant disease of the liver.

The test is correlated to a great extent with changes in the ratio of serum albumin to globulin, but the correlation is not absolute and the mechanism and cause of the reaction remain unexplained.

It has some slight value in prognosis, but its true significance in this field has not been established. It appears to have greater value in establishing the general nature of an hepatic injury—whether it is of extrahepatic or intra-hepatic origin—although more information in this regard is needed.

The many modifications of the technique of the test and different methods of interpreting its results not only make the performance of it confusing, but also cast great doubt on the oft-repeated statement that the test is a simple one.

If the test is to survive there is great need for the establishment of a standard procedure based on a long and varied experience with the test. A careful reading of the literature, however, does not suggest that it would be worth the effort.

61. Rathery, F., and Ferroir, J.: La réaction de Takata; sa valeur diagnostique et pronostique dans les affections du foie, *Paris méd.* 2: 83, 1936.
62. Recht, Stephan: Über die Theorie der Takata-Reaktion, *Klin. Wehnschr.* 15: 1006, 1936.
63. Rohrer, Christian: Über Takata-Ara-Reaktion (Modifikation Jezler) bei Leberaffektionen, *Ztschr. f. klin. Med.* 123: 637, 1933.
64. Schindel, Leo: Zum Mechanismus der Reaktion nach Takata-Ara, *Klin. Wehnschr.* 13: 221, 1934.
65. Schindel, Leo, and Barth, Erich: Die Bedeutung der Takata-Reaktion für die Diagnose der Lebererkrankungen in ihrem Verhältnis zur Galaktose- und Bilirubin-Belastung, *Klin. Wehnschr.* 13: 1329, 1934.
66. Schmengler, F. E.: Über die Takata-Reaktion bei Lungentuberkulose. Ein neuer Beitrag zur Frage der allergischen Entstehung von Hepatopathien, *Klin. Wehnschr.* 18: 742, 1939.
67. Schreuder, J. T. R.: Der Mechanismus der Takata-Ara-Reaktion, *Klin. Wehnschr.* 15: 630, 1936.
68. Seiler, Johannes, and Rehm, Maria: Über die Takata-Reaktion im Blutserum, *Ztschr. f. d. ges. exper. Med.* 100: 736, 1937.
69. Skouge, Erling: Der Wert der Takata-Ara-Reaktion und der Koagulationsbandbestimmung bei Leberkrankheiten, *Klin. Wehnschr.* 12: 905, 1933.
70. Sommer, Johannes: Unsere Erfahrungen mit der Takata-Reaktion an 585 Fällen von Gelbsucht, *München. med. Wehnschr.* 2: 1979, 1937.
71. Sparehez, T., and Vieu, E.: Takata-Ara-Reaktion und ihr klinischer Wert in der Diagnose der, *...* 23, 1939.
72. Staub, H.: Ueber, *...* Wehnschr. 59: 308, 1920.
73. Staub, H., and J Beitrag zum Mechanismus der Takata-Reaktion, *Klin. Wehnschr.* 14: 1635, 1935.
74. Storz, Hans, and Schlunghaus, Hans: Ein klinischer Beitrag zur Pathologie der Blutgerinnung, *Klin. Wehnschr.* 12: 184, 1933.
75. Takata, Maki: Über eine kolloidchemische Sero-diagnostik der Lungenentzündung. Tr. Sixth Congress, Far Eastern Association of Tropical Medicine 1: 693, 1925.
76. Takata, Maki: Ueber die Takata Reaktion in Blut, Eine monographie, Kobe, 1935.
77. Takata, Maki, and Ara, K.: Über eine neue kolloidchemische Liquorreaktion und ihre krastischen Ergebnisse, Tr. Sixth Congress, Far Eastern Association of Tropical Medicine 1: 667, 1925.
78. Taran, Albert, and Lipstein, Samuel: The Takata-Ara Reaction in Amyloidosis, *J. Lab. & Clin. Med.* 24: 479, 1939.
79. Tumen, Henry, and Bockus, H. L.: The Clinical Significance of Serum Proteins in Hepatic Diseases; Compared With Other Liver Function Tests, *Am. J. M. Sc.* 193: 788, 1937.
80. Ueko, H.: Zum Mechanismus der Reaktion nach Takata-Ara Bemerkungen zu der gleichnamigen Arbeit von Schindel in dieser Wochenschrift 1934, 221. *Klin. Wehnschr.* 14: 468, 1935.
81. Ueko, H.: A Serum Test for the Diagnosis of Liver Disturbances, *Guy's Hosp. Rep.* 86: 166, 1936.
82. Ueko, H.: Untersuchungen zum Mechanismus der Takata-Reaktion (Leber- und), *...* Wehnschr. 15: 1074, 1936.
83. Viga L.: La reazione di Takata nelle malattie del fegato,
84. Waybalm, L., and Cherry, C. B.: Takata Reaction in the Blood Serum, *Am. J. Digest. Dis.* 5: 231, 1938.
85. Weiner, S. B.: Takata-Ara Reaction in Various Diseases in Childhood, *J. Mt. Sinai Hosp.* 5: 40, 1938.
86. Wuhmann, F., and Leuthardt, P.: Takata-Reaktion und Bluteiweissfraktionen, *Klin. Wehnschr.* 17: 409, 1938.
87. Zadek, Ernst, Tietze, Albrecht, and Gebert, Karl: Zur Wertung der Leberfunktionsprüfungen am Krankenbett, *Klin. Wehnschr.* 12: 60, 1933.
88. Zirm, K. L.: Über die Beeinflussung der Takata-Ara-Reaktion durch Heparin, *Klin. Wehnschr.* 12: 1693, 1933.

A DISCUSSION OF ELECTROPHORESIS WITH SPECIAL REFERENCE TO SERUM AND ALLERGENS*

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RECENT developments in the methods for the separation of proteins by the moving boundary technique have extended the field of electrophoresis to so great an extent that any attempt to treat the data comprehensively in a short article would be unsatisfactory. We shall, therefore, here outline briefly the theory of electrophoresis, its history, method, and some of its usefulness in studying the components of serum and of ragweed extracts.

ELECTRICAL CHARGE OF PROTEINS

The most striking characteristic of the electrical polarity of protein molecules and protein surfaces is the fact that there are large numbers of amino and carboxyl radicals which act as if they were on the surface.¹ These groups, that is, the COO^- and NH_3^+ groups, serve to determine the net charge of the protein under ordinary conditions. Thus, since protein molecules carry electrical charges, they will migrate when subjected to an electrical field, the direction and velocity depending upon the sign and quantity of the net charge. The molecule is constantly taking on and giving up hydrogen ions, so that the instantaneous charge is a small integral number; but the statistical nature of the charging process allows the time average of the resultant charge to be fractional. Thus, if hundreds of ions are going on and leaving a molecule every second, the instantaneous net charge may be 1, 2, 3, 4, 5, 6, or more whole charges, but the statistical result may be fractional. The surface of the protein molecule is so large and the dissociation constants of its amino acids are so close together that each protein ion acts as if it bore a fractional charge when its mobility in an electrical field is observed. It is quite correct to state, for example, that under given conditions an egg albumen ion may have a net charge equivalent to 4.53 electrons. The fractional nature of the net charge on these large ions makes possible a wide variety of electrophoretic identifications at a given pH.

ISOELECTRIC POINT OF PROTEINS

Whenever the sum of the negative charges on a protein molecule is equal to the sum of its positive charges over a time average, the net charge is zero and the electrical mobility is zero. The protein or the protein surface is called isoelectric. The isoelectric point, therefore, is most difficult to determine directly. At best it can be determined accurately only by interpolation, because

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methods for determining the isoelectric point directly are not sufficiently sensitive to show the small movement which, for example, would be given by a net charge equivalent to 0.01 electron.

One frequently finds the expression "isoelectric range" in connection with proteins like insulin and casein. Although the zone of insolubility of casein may extend over a wide pH range, its isoelectric point, as well as that of insulin, is sharp. Indeed, the rate of change of the electrical charge or of the electric mobility with pH is not accurately represented by the insolubility curve in the zone of the isoelectric point. It lies *somewhere* near the center of this curve, but calculations designed to determine the isoelectric point of proteins from the zone of insolubility are difficult. The isoelectric point should be taken directly from mobility-pH curves. The term "isoelectric range" and similar expressions should be replaced by the "zone of insolubility near the isoelectric point" or similar expressions which refer to the experimental technique used to determine physical characteristics of the protein in question.

EARLY EXPERIMENTS ON SEPARATION

As long ago as 1912 Michaelis and Davidsohn² found that the isoelectric point of hemoglobin under certain conditions was pH 6.8. This point was unaffected by the addition of 1 per cent of serum albumin to the hemoglobin solution; that is, each protein migrated essentially independently of the other. The fact that proteins may exist in solution independent of one another and irrespective of the values of their net charge is well borne out by the behavior of proteins in serum. Aside from these early experiments of Michaelis little attention was given to this aspect of protein chemistry up to the work of Tiselius in the last decade.³ Speaking generally, mutual precipitation of charged colloids is observed only when at least one of the substances is easily precipitated by electrolytes. In harmony with this, Tiselius found that mixtures of proteins of opposite sign and of great difference in net charge were usually stable, although this was not always the case. Tiselius studied the effect of Bence-Jones protein on phycoerythrin and found that the change in the electrical mobility of the two proteins in mixtures was not marked, although their charges were of opposite sign. Using ultraviolet photography to bring out the boundary of the protein in a U-tube of somewhat conventional design, Tiselius found that the boundaries of uniform proteins in their artificial mixtures were reasonably well defined. Since these early experiments by Tiselius have been definitely superseded by his own experiments and by those of investigators who have used his technique, they will not be cited in detail here. The reader interested in the history of the subject should read the appended references.⁴⁻⁶

We must not forget that the microscopie method of electrophoresis may also be used to separate particles having different electrical charges.⁷ Thus, one of us (H. A. A.) has been able to distinguish between the blood cells of different animals using the method of electrophoresis and has repeatedly studied the purity of crystalline precipitates by examining their suspensions in the microelectrophoresis cell. By this method insoluble impurities which have different electrical mobilities may be readily observed. In particular may be mentioned the

gradually a plunger by means of a clockwork into the buffer tube toward which the migration is progressing, or by flowing fresh buffer into the same buffer tube with a mechanically controlled syringe. This tends to raise the level of the liquid on the same side, and since the system seeks hydrostatic equilibrium, there is a flow through the U-tube to the other side. A modification in which one buffer tube is closed has been used.¹⁰ In this case compensation is achieved by forcing buffer either into or out of the closed side.

A single-sectioned tall cell suggested by Tiselius and used in this laboratory (Columbia), schematically shown in Fig. 2, effectively increases the length of the U-tube. The components can migrate the whole length of the tube instead of only half of it, as in the case of the divided cell (Figs. 1 and 4). The bottom section and one leg are filled with the protein solution, while the other leg is filled with buffer. The absence of the middle plates allows complete visibility, so that no boundaries may be obscured. This cell is used only for analytical purposes and not for recovering separated material.

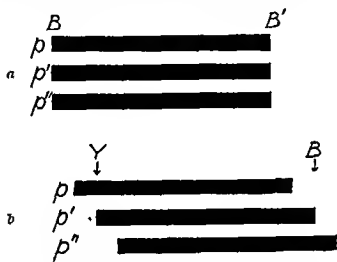


Fig. 3.—*a*, Three component mixture in a horizontal system; *b*, an electric field has acted on the three component mixture.

Before discussing the optical method adapted by Tiselius for observing colorless boundaries, the electrophoresis of a three-component mixture will be considered. In Fig. 3*a* let the three horizontal lines be a mixture of proteins in a horizontal system (for simplicity), forming boundaries with the buffer solution at points marked *B* and *B'*. Let protein *p* have an electrical mobility of one unit, protein *p'*, two units, and *p''*, three units. Let a uniform electric field be applied. After a given period the components will be moved to a position shown in Fig. 3*b*. It is evident that by making a segregation at *Y* and at *B*, a portion of *p* and of *p''* can be obtained in pure form. It is observed, however, that at different points in the system there will be mixtures of *p* and *p'*, *p*, *p'*, and *p''*, and *p'* and *p''*, and that *p'* cannot be obtained in pure form at all. If one starts with the three constituents in a U-tube instead of a horizontal tube, the same phenomena occur. In Fig. 4, *a* shows the protein solution before migration has begun; *b*, after the components have begun to migrate; and *c*, after compensation has pushed the boundaries to the ideal position for separating pure *p* and pure *p''*.

The visualization of colorless boundaries was accomplished in the following way: Advantage was taken of the fact that wherever there are differences

of concentration of protein in solutions there will be a refraction gradient, because the greater the concentration of protein the smaller the velocity of light. Deflection of light passing through these zones of refraction gradients may be detected by an adaptation of an old method of observing defects in telescope lenses. A boundary with sufficient protein acts as if it were a defect in the lens system, and these defects may be photographed and the position of the boundary may thus be marked. In addition, by suitable optical equipment, the concentrations of the materials forming the boundary may be obtained.

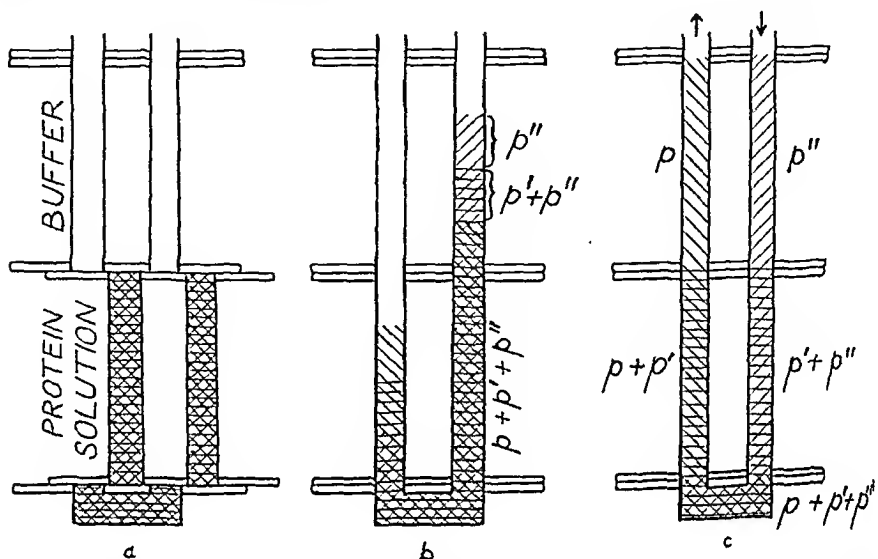


Fig. 4.—*a*, The protein solution before migration has begun; *b*, after the components have begun to migrate; *c*, after compensation has pushed the boundaries to the ideal position for separating pure *p* and pure *p*."

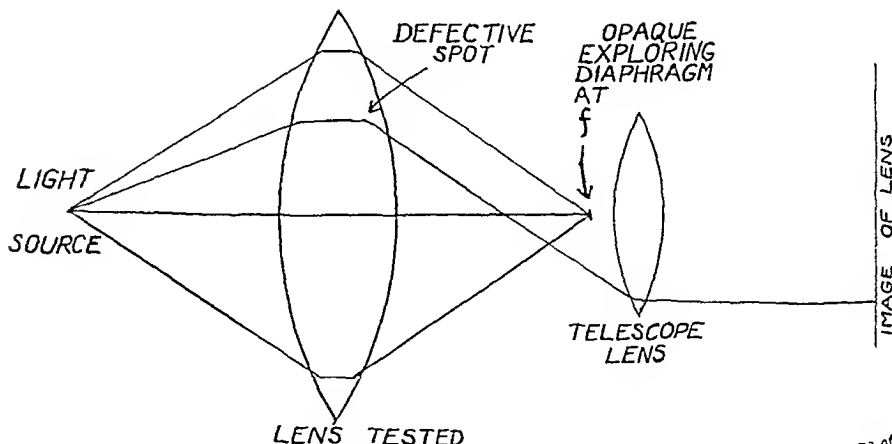


Fig. 5.—Diagram illustrating method of detecting defective spots in lens by means of an opaque, exploring diaphragm. In practice the exploring diaphragm intercepts the deviated rays. For explanation see text.

The optical basis of the schlieren or streak method is described in great detail in many places and suitable references are appended.¹¹ It is dependent upon a method of Foucault,* who devised an accurate technique to test lenses

*This method was originally used by Foucault to test telescope mirrors.

for chromatic and spherical aberration. Foucault employed light from a distant source which he brought to a focus by the lens to be tested (Fig. 5). A diaphragm is placed so that it just covers the image formed at f , the focal point of the lens. An observer looking through a telescope behind the diaphragm and focusing on the lens observes only the rays which fail to converge to a single point at f . These rays which do not strike the diaphragm come from defective portions of the lens and their position is imaged in the telescope. This procedure was reversed by Toepler who by intercepting the deviated rays made the defective portion of the lens appear dark in the telescope. The present method used for detecting concentration gradients in the electrophoresis cell is an adaptation by Tiselius of the Toepler method. If the lens is replaced by the protein solution, the protein gradients at the boundaries appear as defects, giving dark bands where the boundaries occur. Of course, there must be a converging lens (schlieren) just behind the cell containing the solution to bring the rays to a focal point. The walls of the cell, the converging lens, and telescope lens must be without defects in order to have true representation of the boundaries.

APPLICATION TO SERUM PROTEIN

The history of the analysis of serum contains numerous attempts to fractionate serum into constituent proteins ever since serum albumin was crystallized by Guerber.²² Although serum could be separated into several fractions which had different solubilities, these fractions did not fulfill the criteria required to establish them as chemical individuals. Thus Sørensen conceived of serum as a system composed of reversibly dissociable components. Indeed, it was felt that many fractions isolated from serum may have been the results of the treatment of the serum during their isolation. Recently Block²³ came to the conclusion that "serum does not contain several independent proteins. The fractions isolated by physical chemical methods are not pre-existent in serum but result from the technique employed." Although the ultracentrifuge (Svedberg and others) gave much valuable information regarding the nature of serum and its proteins in normal and pathologic conditions, it remained for Tiselius to provide a simple means of attack capable of removing the fractions of serum protein after their separation. It was found, using the method of Tiselius, that serum placed in an electric field without preliminary salt precipitation separates into zones of different electrical mobilities. These components could be isolated and were homogeneous in behavior. In Fig. 6 serum albumin is observed to have the greatest electrical mobility, and instead of two globulins as were commonly supposed to exist in serum, three globulins are found. These are called alpha, beta, and gamma globulin in the order of their electrical mobilities. Similar patterns are observed in horse, human, rabbit, and other sera. In order to get the most clear-cut results, the experiments are performed with serum diluted approximately three times. Once isolated by electrophoresis, the separate constituents of serum exhibit electrical mobilities which are essentially the same as those found in the serum itself. This is important evidence that the individual constituents are present in the serum and does not support the theory that serum is a continuous mixture.

NORMAL AND PATHOLOGIC SERUM

A comparison of the protein composition of normal and pathologic sera has been made by Longworth, Shedlovsky, and MacInnes, as well as by others using the technique of Longworth.¹⁴ In Fig. 6 are shown their electrophoretic diagrams of normal serum and sera of patients with pneumonia, peritonitis, rheumatic fever, peritonsillar abscess, and acute lymphatic leucemia. It will be noticed that all of these diseases exhibit an increase in globulin as determined by the areas under the curves representing the different proteins.

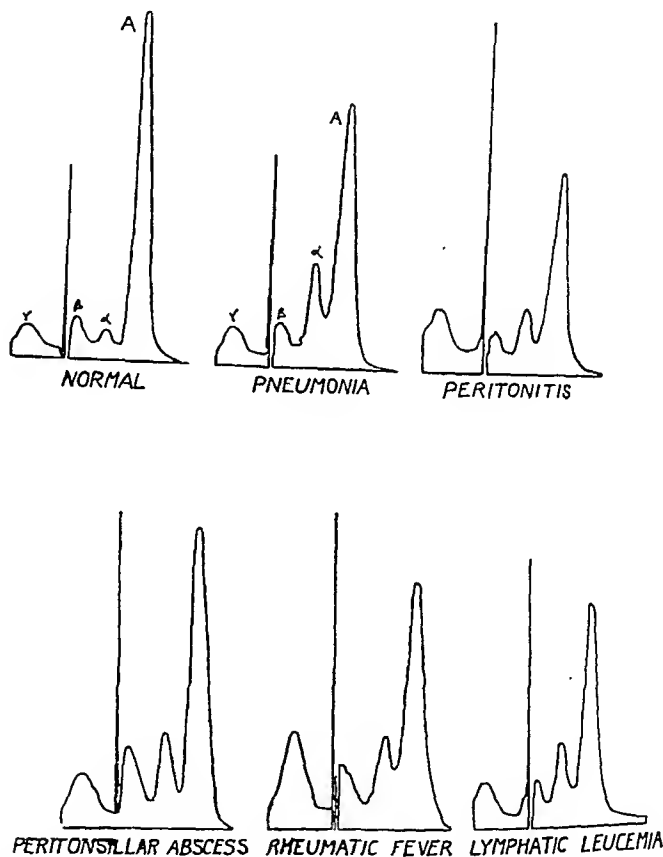


Fig. 6.—Longworth diagrams showing the various constituents of sera in different diseases. These diagrams are copies of similar illustrations published by Longworth, Shedlovsky, and MacInnes.

ELECTROPHORESIS OF ANTIBODIES IN ALLERGY

Newell, Sterling, Oxman, Burden, and Krejci¹⁵ have separated antibodies from allergic serum by the method of electrophoresis. Euglobulin and pseudoglobulin prepared by the older methods are mixtures of globulins isolated by electrophoresis. Euglobulin is essentially the beta and gamma globulin, while pseudoglobulin consists of alpha and gamma globulin. Antibodies, in general, have been found particularly in the pseudoglobulin fraction of the serum corresponding to the alpha and gamma fractions. This was found to be the case for the skin-sensitizing antibody by Sherrer¹⁶ and by Stull, Glidden, and Lovelless¹⁷ by fractional precipitation of pseudoglobulin. Newell and co-workers kept

their serum as concentrated as possible and dialyzed for two days against physiologic saline containing 0.02 M phosphate for buffer. The pH was about 8.1. By collecting and analyzing the separated gamma and beta globulin, it was found that the skin-sensitizing antibody was in the gamma globulin fraction of serum. To check these results serum fractions were examined, using similar techniques for antitoxin against staphylococcus hemolysin. In eight experiments the antitoxin was found only in those fractions which contained gamma globulin. The distribution, therefore, was the same as for the skin-sensitizing antibody.

Sera from three rabbits which had been sensitized by Newell and others with alum-precipitated ragweed extracts were cooled and tested for the precipitin reaction with concentrated ragweed solution. The supernatant liquid, following precipitation, was separated in the Tiselius apparatus, and the upper positive and upper negative fractions were tested against ragweed dilutions. Tests showed that the gamma fraction contained an antibody to ragweed, whereas the upper positive arm contained the faster moving albumin, alpha and beta globulins which were inactive. In rabbits, therefore, it was concluded that the antibody for ragweed is in the same protein fraction as that found by others for anti-egg albumen antibodies.

ELECTROPHORESIS OF ALLERGENS

Extracts of pollen allergens are colored solutions containing pigment and biologically active factors, the nature of which has not as yet been accurately determined because monodisperse systems have not been isolated; that is, there is no evidence that the single chemical substances responsible for hay fever have been isolated as yet by chemical methods. By electrophoresis, however, it is possible to come closer to a solution of the problem. Early experiments to determine the electrical nature of the active constituents of ragweed extract were published some time ago. It was found that the isoelectric point of quartz particles treated with freshly dialyzed ragweed solution was at pH 3.9, with this point shifting to pH 4.3 as the solution stood. At the same time experiments were begun using the conventional moving boundary method to study the electrical charge of the pigment, and to isolate, if possible, the biologically active constituent. With the development of the Tiselius technique the new method lent itself admirably to the separation problem. In Fig. 7, *a* is a diagram obtained by the Tiselius method and the Longworth scanning technique. The US (unpigmented, slow) peak¹⁹ is a colorless constituent which is highly skin reactive. Note in Fig. 7*b* that minor pigmented fractions are present which migrate with velocities faster than the dominant US fraction. The US fraction is highly skin reactive and in certain experiments on skin reactive individuals a solution containing 0.0003 mg. of protein nitrogen per cubic centimeter gave a positive scratch test. Recent unpublished experiments (Fig. 7) with H. Gettner have disclosed that even better fractionation may be obtained using undialyzed extract. We wish to report here in a preliminary way on these experiments. Longworth patterns obtained with the Tiselius technique of undialyzed giant ragweed extract indicate that the colorless constituent previously reported is a dominant probably monodisperse material.

The isoelectric point of this material, as well as the mobility, apparently vary with pH very much the same way that the ragweed covered quartz particles did in the earlier experiment. These data will be reported elsewhere.

The electrophoretic analysis of the pollen extract opens an interesting field for research. It is desirable that the grass pollens be investigated as soon as possible to ascertain whether the immunologic specificity which overlaps among the grasses also is manifested in the chemical behavior of the biologically active constituent separated by the electrophoretic method.

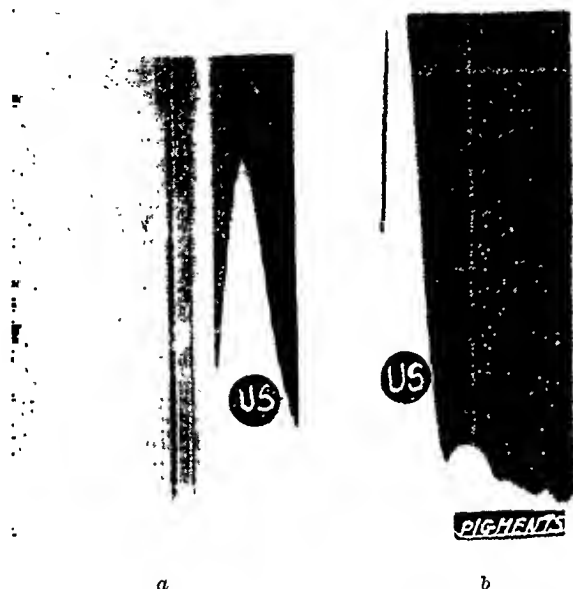


Fig. 7.—*a*, Electrophoretic pattern obtained with undialyzed ragweed extract. The major constituent is an unpigmented, slow moving (US) fraction which is also observed in dialyzed extracts. The gradation in shading depends upon the presence of pigments. Note the absence of pigment where the US fraction is being left behind by the pigments which migrate more quickly.

b, Another sample of undialyzed ragweed extract after one hour and thirty minutes. Electrophoretic pattern to illustrate presence of four pigments in a sample of undialyzed ragweed extract. The US fraction is the major component on the extreme left. Four minor components, faster moving pigments, are found at the lower part of the pattern. In general, six pigments may be observed.

The fractionation of ragweed pollen lends itself to further study of antigen-antibody reaction in atopic sera; that is, it would be of interest to investigate in detail the reaction of the US fraction, as well as the pigment fractions, with allergic sera to see whether the results obtained by Newell and co-workers with crude ragweed extract may be confirmed. It is conceivable that the presence of pigments inhibits or alters an allergen-reagin reaction.

Preliminary data obtained with the US fraction in the ultracentrifuge indicate that its molecular weight is low, probably less than 10,000.

SUMMARY

The nature of the charging process of protein molecules leads to statistical values of the net charge. The large number of differences in the value of the net charge makes the electrophoretic technique desirable for purposes of separation of proteins from one another. This procedure has been greatly improved by the development of the Tiselius moving boundary technique. The applica-

tion of the Tiselius technique to a study of the components of sera, to the properties of allergic antibodies, and to the fractionation of ragweed pollen extract is briefly outlined.

REFERENCES

1. Abramson, H. A., Gorin, M. H., and Moyer, L. S.: *Chem. Rev.* 24: 345, 1939.
2. Michaelis, L., and Davidsohn, H.: *Biochem. Ztschr.* 39: 496, 1912; 41: 102, 1912.
3. Tiselius, A.: *Dissertation, Nova Acta Regiae Soc. Sci. Upsaliensis, Upsala* 4: 7, 1930.
4. Tiselius, A.: *Tr. Faraday Soc.* 33: 524, 1937; *Biochem. J.* 31: 1464, 1937.
5. Longworth, L. G., and MacInnes, D. A.: *Chem. Rev.* 24: 271, 1939.
6. Abramson, H. A.: *Electrokinetic Phenomena and Their Application to Biology and Medicine*, New York, 1934, Chemical Catalog Co., p. 137.
7. Abramson, H. A.: *loc. cit.*, p. 256.
8. Ottenberg, R., and Stenbuck, F.: *J. Gen. Physiol.* 9: 345, 1925.
9. Theorell, H.: *Biochem. Ztschr.* 278: 291, 1935; also earlier papers.
10. Longworth, L. G., and MacInnes, D. A.: *Chem. Rev.* 24: 271, 1939.
11. Schardin, H.: *Das Toeplersche Schlierenverfahren*, Berlin, 1934.
12. Lamm, O.: *Nova Acta Reg. Soc. Sci. Upsaliensis, Upsala*, 1937.
13. Guerher, A.: *Sitzungsb. d. phys.-med. Gesellsch. zu Würzh.* 1: 43, 1894.
14. Block, R. J.: *The Chemistry of Amino Acids and Proteins*, Edited by C. L. A. Schmidt, Springfield, 1938, C. C. Thomas, pp. 312, 329.
15. Longworth, L. G., Shedlovsky, T., and MacInnes, D. A.: *J. Exper. Med.* 70: 309, 1939.
16. Newell, J. M., Sterling, A., Oxman, M. F., Burden, S. S., and Krejci, L. E.: *J. Allergy* 10: 513, 1939.
17. Sherrer, E. L.: *J. Allergy* 2: 467, 1931.
18. Stull, A., Glidden, M., and Loveless, M.: *J. Allergy* 7: 333, 1936.
19. Abramson, H. A., Sookne, A., and Moyer, L. S.: *J. Allergy* 10: 317, 1939.
20. Abramson, H. A., Moore, D. H., Gettner, H., Gagarin, J., and Jennings, L.: *J. Am. Chem. Soc.* 62: 162, 1940.
21. Abramson, H. A., Moore, D. H., Gettner, H., Gagarin, J., and Jennings, L.: *Proc. Soc. Exper. Biol. & Med.* 44: 311, 1940.

RECENT PROCEDURES APPLICABLE TO PROBLEMS IN LIPID METABOLISM

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DURING the existence of this JOURNAL abundant evidence for the fact that progress in clinical investigations often follows advancement in analytical procedure has been presented. It seems fitting, therefore, in contributing to this, the Silver Anniversary Number, to call attention to some of the newer methods of attack applicable to studies in lipid metabolism.

The early issues of this JOURNAL contain accounts of the current methods for lipid analysis. While considerable information was obtained as a result of the use of these procedures, the knowledge gained was nevertheless incomplete because of the shortcomings of the particular methods employed. The importance of the phospholipids in fatty acid transport was demonstrated, and while it was suspected that one of these, lecithin, probably played a more important role than the others, such a conclusion was not warranted because at that time the available method was limited to the determination of the total phospholipids only. Nevertheless, it was usually stated that lecithin was the vehicle most intimately concerned in this transport. In addition to this, no method was available for the direct determination of the small amounts of neutral fat of the blood, nor was it possible to ascertain the nature of the acids combined with neutral fat or cholesterol. Recent advances have been made which should assist in the clarification of these problems.

Three methods have recently been described whereby a very satisfactory fractionation of the phospholipids of the blood can be accomplished. These methods are of such a nature that it is not necessary to employ a highly trained chemist for their use, since with some practice they should be handled by the well-trained laboratory technician. The methods are similar in that in each case a quantitative determination of choline is made. Lecithin and sphingomyelin contain approximately equal amounts of this base, whereas none is present in cephalin. Thus by determining total phospholipids and choline the cephalin content can be calculated. Williams and co-workers,¹ as well as Kirk,² rely on the insolubility of sphingomyelin in moist ether for its determination, whereas Thannhauser and others³ precipitate the compound with Reinecke's acid. These are distinct advantages over the older procedures which depended entirely upon fractionation with various fat solvents. On the whole, the Thannhauser procedure seems to be the most desirable of the three because of the direct determination of the sphingomyelin, but it requires as much as 60 c.c. of plasma or cells. A micromethod, based to some extent on the Thannhauser procedure, has been developed in the Research Laboratory of the Children's Fund of Michigan. This procedure should be in press in the near future and should be a valuable contribution, inasmuch as only 3 c.c. of plasma are required. Thannhauser has

reported that the sphingomyelin content of normal human serum comprises about 10 per cent of the total phospholipids, while cephalin³ and lecithin are present in approximately equal amounts (45 per cent). Data on pathologic blood specimens have as yet not been reported, but the method has been successfully applied to normal human organs.⁴ As Thannhauser points out, "It will be of interest to observe whether the values for the various phospholipids vary in their proportion in organs under pathological conditions, especially in so-called fatty degeneration." Very recently Artom and Freeman,⁵ using a procedure consisting of a combination of the Kirk and Thannhauser methods, determined the individual phospholipids in the plasma of rabbits after a fatty meal and observed that a definite increase in the total phospholipids was only occasionally observed. When this took place, higher values for lecithin were generally in evidence, but lower cephalin values were usually encountered when the fat was fed. These data indicate that lecithin was the only phospholipid concerned with the transportation of the fatty acids. The old view that this was the case now rests on a more secure foundation.

In the past the blood phospholipids had been assigned another important role. In 1919 Meigs, Blatherwick, and Cary⁶ proposed that these lipids were the precursors of the fat of milk. At that time this appeared to be an attractive theory, but reinvestigations of the problem by others⁷ threw much doubt upon the proposal. Recently the application of a newly developed method for neutral fat, originated by Voris, Ellis, and Maynard⁸ has offered support to an alternate and more tenable view. Blood was taken from the subcutaneous abdominal vein and iliac artery of the lactating cow and analyzed for neutral fat. The results obtained indicate that a removal of neutral fat takes place during the circulation of blood through the active mammary gland. This fraction of the blood lipids thus contributes to the formation of milk fat. The gland itself must, however, also contribute to this phenomenon, since the simple removal of neutral fat from the blood stream cannot account for the peculiar composition of fatty acids of low molecular weight. As is the case with the other procedures discussed, the method has only been applied to a small extent, but it should prove to be very useful in the future.

Another recent procedure which should have great possibilities is one devised by Kelsey⁹ for determining the fatty acids in the different blood lipid fractions. The phospholipids are removed by precipitation with acetone, and residual lipids are digested with a lipase obtained either from pancreas that had previously been treated with ammonia in order to destroy the cholesterol esterase, or from the castor bean. Under such conditions only the neutral fat is split and the fatty acids thereby liberated can be isolated. Those acids combined with cholesterol can be freed by saponification. Few applications have as yet been made of the procedure, and some trouble has been encountered in its application to the blood. This difficulty will probably be overcome. The future should bring valuable information from the use of the method, thus increasing our knowledge of the types of fatty acids transported by the different constituents of the blood lipids under normal and abnormal conditions.

Some of the most valuable data on lipid metabolism have recently been obtained as the result of the use of isotopes or radioactive elements for labeling substances synthesized or formed in the normal living organism. Outstanding in the use of deuterium, the isotope of hydrogen, are the investigations of Schoenheimer and his associates, whose work has recently been reviewed.¹⁹ These investigators have clearly demonstrated that a continuous synthesis of cholesterol and fatty acids takes place in the animal organism. In following the fate of various labeled fatty acids, it was demonstrated that those of low molecular weight disappeared almost explosively, whereas those of high molecular weight were, to some extent at least, deposited in the tissues. These observations are in accordance with those obtained by others who had fed the naturally occurring fatty acids. In addition to this it was observed that a conversion of saturated to unsaturated acids, and vice versa, also took place in the normal intact animal. Palmitic acid was reported as being transformed into homologues of higher and lower molecular weight, as well as into derivatives containing one double bond. Although a synthesis of oleic was clearly demonstrated, no indication that linoleic acid, Burr's essential fatty acid, could be formed *de novo* was achieved. Cetyl alcohol, a constituent of normal feces, was also found to be synthesized in vivo and was proved to arise from palmitic acid. When this alcohol was labeled with heavy hydrogen and fed to rats, deuteropalmitic acid, as well as deuterostearic acid, was isolated. This alcohol was thus assigned the role of an intermediate between these two acids, and it was suggested that other fatty acids might be formed similarly from other alcohols. While many transformations referred to above have been suggested by the work of others, Schoenheimer's data are probably the first clear-cut demonstration of their occurrence in the normal intact animal organism.

The phospholipids have not been overlooked by those interested in this new method of investigation. This phase of the question has been reviewed several times by Hevesy, his latest contribution appearing in the *Annual Review of Biochemistry* of 1940.¹¹ Chaikoff and his co-workers at the University of California have studied the phospholipid metabolism of different tissues of the white rat and have observed that a formation of new phospholipids takes place almost continuously in all the tissues investigated.^{12, 14} The activities of the tissues as far as this phospholipid turnover is concerned were ranked in the following order: liver and small intestine, very active; kidney, moderately active; stomach and large intestine, slightly active; and central nervous system, sluggish. In many respects these results are in accordance with the conceptions of Sinclair¹⁵ who employed elaidic acid as a tool for following lipid metabolism. Chaikoff¹³ has also demonstrated that the turnover in the liver is independent of the one taking place in the gastrointestinal tract, since it occurs even when that whole tract is removed. This emphasizes anew the importance of the liver in fat metabolism. These same workers have also observed that this turnover in the liver can be accelerated by the administration of choline¹⁶ and depressed by cholesterol.¹⁷ Such data offer an explanation of the fact that whereas fatty livers can be produced by feeding diets rich in cholesterol,¹⁵ the administration of choline¹⁹ prevents the accumulation of fat. Betaine has likewise been found to exert a lipotropic action,²⁰ and like choline it has been shown

to stimulate the turnover of phospholipids in the liver.²¹ In their most recent work the California investigators²² report that the amino acid methionine has a similar effect on the hepatic phospholipids. This was to be anticipated in the light of the demonstration²³ that this amino acid, like choline, prevents the formation of fatty livers of rats on low protein-high fat diets. In contrast to this, cystine, the other naturally occurring sulfur-containing amino acid, actually causes an increase in liver fat.²⁴ It was indeed surprising to find in this latest report by Chaikoff that cystine, like methionine, also stimulates the phospholipid turnover in the liver. This observation makes it difficult to explain the opposite effects on the total liver lipid contents that have been almost regularly observed when these acids were employed separately as supplements to low protein-high fat diets. It would be of interest to determine what influence Dragstedt's "lipocaine" would have upon this turnover. According to Dragstedt,²⁵ the action of lipocaine on fatty livers is different from that of choline, and if this is the case, perhaps some additional information can be obtained by the use of radioactive phosphorus. To date, Chaikoff and his co-workers have not fractionated the phospholipids, and it is impossible to state which of these were labilized as a result of the administration of choline and allied substances. In the light of the work of Chargaff,²⁶ however, it is probable that the lecithin fraction is the more labile one. Chaikoff and his aides²⁷ have determined the total phospholipid turnover of four different tumors transplanted into mice and report that the speed of this reaction resembles that of the more active tissues as the liver and kidney rather than that of the more stable muscles and brain.

In concluding this discussion it is fitting to call attention to the excellent work on the blood lipids that is being done in the Research Laboratory of the Children's Fund of Michigan. This group has not been content merely to determine the distribution of the lipids in the plasma and erythrocytes but has investigated the platelets and developed a method for the preparation of the stroma suitable for subsequent analysis.²⁸ Detailed studies, including total lipids, individual phospholipids, cholesterol, cholesterol esters, and neutral fat, have been made of the bloods of different species. A close relationship between size of cell and lipid content was shown to exist, the big avian cells containing large amounts of total lipids, the little sheep cells small amounts, with the human erythrocytes intermediate with regard to size and lipid content.²⁹ Almost all the lipids in the corpuscles of mammalia were found to be segregated in the stroma, indicating that these lipids are not concerned with the transport of fatty acids, but play a structural role in the makeup of the red blood cell.²⁹ Cephalin, which is not generally believed to be as intimately connected with lipid metabolism as is lecithin, was found to be the predominating lipid (45 per cent). The total lipid content, as well as the distribution of the individual phospholipids of the platelets, was found to be essentially like that of the stroma, the phospholipids comprising approximately 75 per cent and primarily in the form of cephalin.³⁰ This lipid has been assigned a role in the phenomenon of blood coagulation, and for this reason platelets obtained from a number of hemophiliacs were examined. Although a delay in disintegration time was clearly demonstrated, no deficiency in cephalin content was observed.³⁰ Thus the hemophiliac is abundantly supplied with cephalin; this raises the question as to

the delayed clotting time. Is the difference due perhaps to a variability of the constituent fatty acids in the cephalin molecules of the hemophiliacs? Unfortunately, micromethods applicable for determining the chemical nature of the fatty acids present in the small amounts of lipids obtainable from the platelets are as yet not available to answer this question. There is a definite need for such a procedure. Platelets obtained during menstruation, as well as those from one case of congenital thrombopenic purpura, were likewise found to be normal so far as lipid content and distribution are concerned.³¹

REFERENCES

1. Williams, H. H., Erickson, B. N., Avrin, I., Bernstein, S. S., and Macy, I. G.: Determination of Cephalin in Phospholipids by the Estimation of Choline, *J. Biol. Chem.* 123: 111, 1938.
2. Kirk, E.: A Micro Method for Approximate Estimation of Lecithin, Cephalin, Ether-Insoluble Phosphatide, and Cerebrosides in Plasma, Red Blood Cells, and Tissues, *J. Biol. Chem.* 123: 623, 1938.
3. Thannhauser, S. J., Benotti, J., and Reinstein, H.: The Determination of Lecithin, Cephalin, and Sphingomyelin in Body Fluids and Tissues; With Analyses of Normal Human Sera, *J. Biol. Chem.* 129: 709, 1939.
4. Thannhauser, S. J., Benotti, J., Walcott, A., and Reinstein, H.: The Lecithin, Cephalin, and Sphingomyelin Content of Human Organs, *J. Biol. Chem.* 129: 717, 1939.
5. Artom, C., and Freeman, J. A.: Individual Phospholipids in Plasma of Rabbits After a Fatty Meal, *J. Biol. Chem.* 133: iv, 1940.
6. Meigs, E. B., Blatherwick, N. R., and Cary, C. A.: Contributions to the Physiology of Phosphorus and Calcium Metabolism as Related to Milk Secretion, *J. Biol. Chem.* 37: 1, 1919.
7. Blackwood, J. H.: The Relationship of Phosphorus to Fat Metabolism, *Biochem. J.* 28: 1346, 1934.
8. Voris, L., Ellis, G., and Maynard, L. A.: The Determination of Neutral Fat Glycerol in Blood With Periodate, *J. Biol. Chem.* 133: 491, 1940.
9. Kelsey, F. E.: The Action of Castor Bean and Pancreatic Lipase on Blood Neutral Fat, *J. Biol. Chem.* 130: 199, 1939.
10. Schoenheimer, R., and Rittenberg, D.: The Study of Intermediary Metabolism With the Aid of Isotopes, *Physiol. Rev.* 20: 218, 1940.
11. Hevesy, G.: Application of Radioactive Indicators in Biology, *Ann. Rev. Biochem.* 9: 641, 1940.
12. Perlman, I., Ruben, S., and Chaikoff, I. L.: The Rate of Formation and Destruction of Phospholipids in the Fasting Rat, *J. Biol. Chem.* 122: 169, 1937.
13. Fries, B. A., Ruben, S., Perlman, I., and Chaikoff, I. L.: The Role of the Stomach, Small Intestine, and Large Intestine in Phospholipid Metabolism in the Presence and Absence of Ingested Fat, *J. Biol. Chem.* 123: 587, 1938.
14. Changus, G. W., Chaikoff, I. L., and Ruben, S.: The Phospholipid Metabolism of the Brain, *J. Biol. Chem.* 126: 493, 1938.
15. McConnell, K. P., and Sinclair, R.: Evidence of Selection in the Building Up of Brain Lecithins, and Cephalins, *J. Biol. Chem.* 118: 131, 1937.
16. Perlman, I., and Chaikoff, I. L.: On the Mechanism of the Action of Choline Upon the Liver of the Fat Fed Rat, *J. Biol. Chem.* 127: 211, 1939.
17. Perlman, I., and Chaikoff, I. L.: The Influence of Cholesterol Upon the Phospholipid Turnover in the Liver, *J. Biol. Chem.* 128: 735, 1939.
18. Okey, R.: Effect of Cholesterol Feeding Under Varying Conditions Upon Lipids of Rat Livers, *Proc. Soc. Exper. Biol. & Med.* 30: 1003, 1933.
19. Best, C. H., and Huntsman, M. E.: The Effect of Choline on the Liver Fat of Rats in Various Stages of Nutrition, *J. Physiol.* 83: 255, 1935.
20. Best, C. H., and Huntsman, M. E.: The Effect of the Components of Lecithin Upon the Deposition of Fat in the Liver, *J. Physiol.* 75: 403, 1932.
21. Perlman, I., and Chaikoff, I. L.: The Influence of Betaine on the Phospholipid Activity of the Liver, *J. Biol. Chem.* 130: 593, 1939.
22. Perlman, I., Stillman, N., and Chaikoff, I. L.: The Influence of Methionine, Cystine, and Cysteine Upon the Phospholipid Turnover in the Liver, *J. Biol. Chem.* 133: 651, 1940.
23. Tucker, H. F., and Eckstein, H. C.: The Effect of Supplementary Methionine and Cystine on the Production of Fatty Livers by Diet, *J. Biol. Chem.* 121: 479, 1937.
24. Beeson, A. W., and Channon, H. J.: Cystine and the Dietary Production of Fatty Livers, *Biochem. J.* 30: 280, 1936.

25. Dragstedt, L. R., Vermeulen, C., Goodpasture, W. C., Donovan, P. B., and Geer, W. A.: Lipocae and Fatty Infiltration and Pancreatic Diabetes, *Arch. Int. Med.* 64: 1017, 1939.
26. Chargaff, C.: The Relative Speed of Formation of Lecithin and Cephalin in the Body, *J. Biol. Chem.* 128: 587, 1939.
27. Jones, H. B., Chaikoff, I. L., and Lawrence, J. H.: The Phospholipid Metabolism of Neoplastic Tissues, *J. Biol. Chem.* 128: 631, 1939.
28. Bernstein, S. S., Jones, R. L., Erickson, B. N., Williams, H. H., and Macy, I. G.: A Method for the Preparation of Posthemolytic Residue or Stroma of Erythrocytes, *J. Biol. Chem.* 122: 507, 1938.
29. Erickson, B. N., Williams, H. H., Bernstein, S. S., Avrin, I., Jones, R. L., and Macy, I. G.: The Lipid Distribution of Posthemolytic Residue or Stroma of Erythrocytes, *J. Biol. Chem.* 122: 515, 1938.
30. Erickson, B. N., Williams, H. H., Avrin, I., and Lee, P.: The Lipid Distribution of Human Platelets in Health and Disease, *J. Clin. Investigation* 18: 81, 1939.
31. Lee, P., and Erickson, B. N.: Platelet Studies in Menstruation and Hemophilia, *Proc. Soc. Exper. Biol. & Med.* 39: 264, 1938.

CLINICAL STUDIES OF THE ORGANIC ACID-SOLUBLE PHOSPHORUS OF RED BLOOD CELLS IN DIFFERENT ACIDOTIC STATES*

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EXCESSIVE phosphaturia in diabetes was noted and extensively studied around 1900 by von Noorden, Mandel and Lusk, and others.¹ In 1907 Fitz, Alsberg, and Henderson² found that hydrochloric acid given to rabbits by stomach tube led to greatly increased excretion of phosphates in the urine. These and other investigations of that period led to an understanding of the role of the urinary phosphates as buffer substances, and of the important mechanism by which the secretion of phosphates serves to conserve the base stores of the body in acidosis.³ For many years thereafter, however, little was learned concerning the physiologic adjustments which could permit or effect such changes in the urinary phosphate excretion.

In 1924 Haldane and his co-workers⁴ found that a reduced concentration of organic acid-soluble phosphorus in the blood cells was associated with large losses of phosphorus in the urine during ammonium chloride acidosis. They pointed out then that some of the bad effects of prolonged acidosis might be due to the depletion of the phosphorus reserves of the body. In 1927 Byrom⁵ found a great reduction in the concentration of the organic acid-soluble phosphorus (ester P) in the blood cells during severe diabetic acidosis. In 1937 Rapoport⁶ identified as diphosphoglyceeric acid the fraction of the organic acid-soluble phosphorus in the blood cells that decreased during ammonium chloride acidosis; and later the diphosphoglyceerate fraction in the blood cells was found to be similarly decreased in diabetic acidosis.⁷ Diphosphoglycerate in the blood appears, therefore, to be one source of the increased amount of phosphorus excreted in the urine in these two types of acidosis.

Contrasted with the phenomenon of increased phosphaturia in these types of acidosis, the excretion of phosphates in the urine was long known to be diminished in conditions with impaired renal function. In such conditions, an increase of inorganic phosphorus in the blood was accorded considerable diagnostic and prognostic importance as a sign of failure of renal function. Marriott and Howland⁸ in 1916 stressed the importance of the *retention* of inorganic phosphates as a factor in the production of nephritic acidosis. While the importance of this factor was debated by many investigators during the succeeding years, little attention was paid to the organic acid-soluble phosphorus compounds of the blood cells. In 1927 Byrom and Kay⁹ found an increased concentration of ester phosphorus in the blood cells of patients with severe nephritis, and in 1934 Ashley and Guest¹⁰ found similar changes in the blood cells of rabbits and

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dogs following the suppression of renal function by bilateral ureteral ligation, mercuric chloride poisoning, and diphtheria intoxication. Later, the increases of organic acid-soluble phosphorus in the blood cells under such conditions were accounted for in the diphosphoglycerate fraction.^{7, 11, 12}

Diphosphoglycerate normally makes up about half of the organic acid-soluble phosphorus of human blood. In a number of conditions, however, its concentration in the blood cells has been found to vary between wide limits: *increased*, after pyloric obstruction, following overdosage with irradiated ergosterol, and in nephrotis; *decreased*, in experimental rickets and in certain acidotic states. In such conditions, the changes in the blood cells probably reflect to a high degree the related and interdependent chemical changes which occur concomitantly in other body tissues. Thus, the concentrations of the acid-soluble phosphorus compounds in the blood, and the speed and direction of the reactions by which they are synthesized and decomposed, may constitute valuable indices of the state of the phosphorus stores and phosphorus metabolism of the whole body.

The data presented here demonstrate changes in the blood which have been found in acidotic states due to four different causes: the ingestion of ammonium chloride, diabetes mellitus, gastroenteritis in infants, and severe nephritis. The discussion that follows is designed to call attention to various factors which may influence such changes, and to emphasize their significance in the chemical pathology of these conditions.

METHODS

The methods used in these investigations are described elsewhere,^{7, 12} except for minor modifications.

In Tables I to IV, data on the phosphorus distribution in different types of blood are correlated with values, determined in the same samples, for the carbon dioxide content of serum, chloride concentrations in serum and cells, and serum pH. Chabanier and Lévy and co-workers¹³⁻¹⁶ have emphasized the value of the ratio between concentrations of chloride in cells and serum ($\text{Cl}_c:\text{Cl}_s$) as a measure of acidosis in clinical diagnosis. While this ratio is primarily dependent upon the pH of the blood, it appears to have additional importance in relation to changes of the organic acid-soluble phosphorus in the blood cells.

AMMONIUM CHLORIDE ACIDOSIS

Data in Table I illustrate changes found in the blood of one of us (S. R.) after the ingestion of 25 Gm. of ammonium chloride by mouth, in divided doses, on two successive days and 12 Gm. the third day. During three days of development of acidosis, the excretion of phosphorus in the urine was greatly increased, as has been observed repeatedly in this type of experiment by other investigators. Data in the two columns of Table I represent the findings on blood samples drawn before the experiment and at the end of the third day. In the latter sample, concentration of the blood is shown by the increased volume and number of red blood cells, and by the increased serum protein. The serum pH fell from 7.37 to 7.06. The carbon dioxide content of the blood decreased

more in the serum than in the cells; and the concentration of chloride increased more in the cells than in the serum. The inorganic phosphorus in the whole blood decreased slightly. The concentration of organic acid-soluble phosphorus in the cells decreased markedly, and this decrease was practically all accounted for by a nearly equal decrease in the diphosphoglycerate.

This experiment has been reported elsewhere, with a chart illustrating intermediate changes found in blood samples drawn during the development of acidosis and during recovery.*

TABLE I

AMMONIUM CHLORIDE ACIDOSIS

DATA ON THE BLOOD OF S. R., BEFORE AND AFTER TAKING 62 GM. OF AMMONIUM CHLORIDE BY MOUTH IN DIVIDED DOSES DURING THREE SUCCESSIVE DAYS

	BEFORE	AFTER
Volume of cells in whole blood (%)	42.9	50.6
Red cell count (millions per c. mm.)	4.99	5.87
Hb in whole blood (Gm. per 100 c.c.)	14.7	18.0
Protein, serum (Gm. per 100 c.c.)	6.6	8.9
pH of serum	7.37	7.06
CO ₂ content, serum (meq. per 1)	28.2	15.8
CO ₂ content, cells (meq. per 1)	18.8	15.3
Cl, serum (meq. per 1)	103.0	109.2
Cl, cells (meq. per 1)	50.6	66.9
Ratio Cl _s :Cl _c	0.49	0.61
Phosphorus (mg. per 100 c.c.)		
Inorganic P, serum	4.2	3.5
Organic acid-soluble P, cells	47.9	32.5
Diphosphoglycerate P, cells	28.4	16.0

TABLE II

DIABETIC ACIDOSIS

DATA ON BLOOD SAMPLES FROM TWO PATIENTS, E. W. AND P. H., DRAWN DURING COMA AND AFTER RECOVERY

	E. W.		P. H.	
	DURING COMA	7 DAYS LATER	DURING COMA	4 DAYS LATER
Volume of cells in whole blood (%)	48.2	29.7	47.1	31.2
Red cell count (millions per c. mm.)	5.50	3.55	4.37	3.48
Hb in whole blood (Gm. per 100 c.c.)	13.7	8.7	12.6	9.8
pH of serum	6.84	7.42	6.87	7.42
CO ₂ content, serum (meq. per 1)	3.8	22.3	5.7	24.3
Cl, serum (meq. per 1)	107.1	100.3	88.5	106.0
Cl, cells (meq. per 1)	80.3	56.5	66.6	51.2
Ratio Cl _s :Cl _c	0.75	0.56	0.75	0.48
Phosphorus (mg. per 100 c.c.)				
Inorganic P, whole blood	4.3	3.1	10.8	2.2
Organic acid-soluble P, cells	22.3	60.0	31.8	55.6
Diphosphoglycerate P, cells	< 3.0	36.4	7.1	29.3

DIABETIC ACIDOSIS

Examples of data on blood samples drawn from two patients in diabetic coma, and after recovery, are presented in Table II. These patients were adults, on the medical service of the Cincinnati General Hospital. Both had been in coma several hours before they were brought to the hospital, when the first blood samples were drawn. The first patient, E. W., is the subject of a chart

*Chart 5.

in another paper,* a chart which shows graphically the progressive changes which were found in the blood samples drawn from this patient at intervals during the recovery period. From a series of similar patients thus far studied, these two are selected for presentation here because of striking differences in concentration of chloride in the blood serum in the two initial blood samples.

In the first samples of blood drawn from both patients, a severe degree of acidosis was indicated by the extraordinarily low values for pH and carbon dioxide content of the serum, and with these changes the concentration of organic acid-soluble phosphorus and diphosphoglycerate in the cells was very low. While the concentration of chloride in the serum was relatively high in the serum of one (E. W.), and low in the serum of the other (P. H.), the concentration of chloride in the cells of both was increased, and the ratios $\text{Cl}_c:\text{Cl}_s$ were very high. The high inorganic phosphorus in the first sample of blood of P. H. can be ascribed to severe dehydration and beginning failure of renal function. After recovery all values returned to within normal limits. The recovery of a normal chemical structure of the blood varies in individual cases, however, and does not necessarily parallel symptomatic recovery.

TABLE III
ACIDOSIS DUE TO GASTROENTERITIS IN THREE INFANTS

	D. C. (AGE, 1 MO.)	J. B. (AGE, 2 MO.)	W. S. (AGE, 7 MO.)
Volume of cells in whole blood (%)	44.1	35.0	48.5
Red cell count (millions per c. mm.)	4.29	3.30	6.2
Hb in whole blood (Gm. per 100 c.c.)	14.8	10.0	16.3
Urea N (mg. per 100 c.c.)			73.0
CO_2 content, serum (meq. per 1)	5.3	4.9	10.8
Cl, serum (meq. per 1)	114.0	119.0	114.0
Cl, cells (meq. per 1)	101.1	90.7	
Ratio $\text{Cl}_c:\text{Cl}_s$	0.89	0.76	
Phosphorus (mg. per 100 c.c.)			
Inorganic P, whole blood	6.3	7.8	2.8
Organic acid-soluble P, cells	30.8	28.2	25.5

ACIDOSIS OF GASTROENTERITIS IN INFANTS

In Table III are listed data on blood samples from three infants, 1, 2, and 7 months of age, respectively, suffering from acute profuse diarrhea, dehydration, and severe acidosis. In a series of cases of gastroenteritis thus far studied, the blood changes illustrated here are typical of the state of acidosis that develops in infants when severe diarrhea is attended by little or no vomiting. Other types of change are found, however, when excessive vomiting, renal impairment, and other factors play variable roles in the disturbance; these will be treated more fully in later communications.

In each case the carbon dioxide content of the serum and the concentration of organic acid-soluble phosphorus in the cells was very low. The concentration of chloride was increased in both serum and cells, and the ratio $\text{Cl}_c:\text{Cl}_s$ was very high, indicating a very low pH. More complete analyses were not made because of the limitations imposed by the small blood samples available.

*Chart 7.

Other investigators have demonstrated, however, that the state of acidosis in this type of disturbance is characterized by a low concentration of total base in the blood serum, i.e., a base deficit due to losses of base in diarrheal stools.^{17,18}

TABLE IV

NEPHRITIS

EXAMPLES OF DATA ON BLOOD SAMPLES FROM PATIENTS WITH VARYING DEGREES OF RENAL IMPAIRMENT AND IN DIFFERENT CONDITIONS OF ACIDOSIS

	1	2	3	4
	E. S.	C. P.	P. G.	R. H.
Total cells in whole blood (%)	15.3	23.6	22.8	14.4
Red cell count (millions per c. mm.)	2.04	2.84	2.47	1.77
Hb in whole blood (Gm. per 100 c.c.)	4.4	7.2	6.3	4.8
Protein, serum (Gm. per 100 c.c.)				6.9
Nonprotein N (mg. per 100 c.c.)	55.0	200.0	76.0	156.0
pH, serum				7.12
CO ₂ content, serum (meq. per l)	14.0	15.8	3.2	14.7
Cl, serum (meq. per l)	103.2	84.5	105.3	103.3
Cl, cells (meq. per l)	49.6	35.8	79.4	70.1
Ratio Cl ₂ :Cl ₁	0.48	0.42	0.75	0.68
Calcium, serum (mg. per l)		2.6	8.2	6.1
Phosphorus (mg. per 100 c.c.)				
Inorganic P, whole blood	9.1	13.5	9.4	7.4
Organic P, cells	79.1	79.4	35.6	59.0
P, cells		60.4	11.0	

NEPHRITIS

Data in Table IV permit comparisons of different patterns of blood changes found in four patients suffering with severe impairment of renal function of varying duration and severity. The data indicating anemia, low carbon dioxide content, and high concentrations of nonprotein nitrogen and of inorganic phosphorus in the blood are characteristic of nephritic acidosis. However, the concentrations of organic acid-soluble phosphorus in the cells, and of chloride in the serum and cells, differed widely among the four blood samples represented here. A tentative interpretation of the significance of these findings is offered in the discussion that follows.

1. *Subacute bacterial endocarditis, hemorrhagic nephritis.* E. S., white female, 15 years of age. Her illness of eight months' duration began with signs of rheumatic heart disease and progressed slowly, with increasing signs of cardiac decompensation, slight edema, hematuria, occasional vomiting, etc. Death occurred with convulsions one week after this blood sample was taken.

2. *Chronic glomerulonephritis.* C. P., colored male, 9 years of age. The duration of illness is uncertain. The boy was first admitted to the hospital two years previously, with acute nephritis associated with edema, albuminuria, elevated blood pressure, and urea clearance of only around 3 per cent. During two years he was readmitted to the hospital several times with exacerbations of the same symptoms. He died two days after this blood sample was taken.

3. *Renal rickets.* P. G., white male, 9 years of age. A diagnosis of congenital malformation of the bladder, hydronephrosis, and renal rickets was made when this boy, at the age of 5 years, was first brought to the hospital because of poor development and poor weight gain, occasional vomiting, and urinary incontinence. His renal function measured less than 10 per cent of phenolsulfonephthalein excretion in two hours. At the time this blood sample was taken, he had been brought to the hospital after five days of increasing illness, apparently precipitated by a mild respiratory infection. He was then very drowsy, breathing

was labored, and the blood showed an extreme acidosis. Following the administration of sodium bicarbonate to correct this acidosis, he showed a remarkable transient improvement of his symptoms, but he died a month later.

4. *Nephrosclerosis*. R. H., white male, 13 years of age. The duration of illness is uncertain. There was a history of increasing weakness, anemia, muscle cramps, etc., since less than 2 years; pus and albumin in the urine were noted by physicians during the same period. The mother reported having seen "strings of pus" in the urine since infancy. He had convulsions during a respiratory infection when 2 years old, but apparently had none during an attack of scarlet fever at 6 years of age. During nearly a year's observation in this hospital he has had several exacerbations of acute symptoms, with vomiting, marked anemia, variable azotemia, and with Trousseau and Chvostek signs occasionally positive. This blood sample was taken at a time when he was feeling comparatively well, although it was deemed necessary to give him a transfusion shortly afterward because of the low red blood cell count and hemoglobin found in this sample.

DISCUSSION

Several recognized functions of the organic acid-soluble phosphorus compounds of the blood cells have been discussed previously in a series of papers based on experimental and clinical studies carried on in this laboratory. These substances participate in carbohydrate metabolism, serve in the transport of phosphorus in the body for various metabolic needs, and play an important role in the maintenance of the acid-base equilibrium of the blood.

In earlier papers of this series attention was called to a reciprocal relationship that was observed between changes in concentration of organic acid-soluble phosphorus and chloride in the blood cells following pyloric obstruction¹⁹ and after suppression of renal function.¹⁰ More recently available data on the several fractions of the organic acid-soluble phosphorus have permitted a fairly complete description of the ionic composition of blood cells, both in the normal state and in several conditions in which severe disturbances of the blood electrolytes are known to occur. When the organic phosphorus compounds are taken into consideration, close agreement is obtained between the total base and the sum of the anions in the cells. Evidence has been presented that the distribution of the diffusible ions, H^+ , Cl^- , and HCO_3^- , between cells and serum depends mainly upon the concentration and anion equivalency of diphosphoglyceric acid and hemoglobin, the two most important nondiffusible constituents of the cells.^{12, 20}

Changes in concentration of the organic acid-soluble phosphorus compounds in the blood cells appear to be subject to several interdependent factors: the functional state of the tissue phosphorus reserves, the excretion of phosphates by the kidneys, the changes in concentration of other electrolytes in the blood; and also, various factors affecting the reactions of the glycolytic cycle by which these phosphoric esters are synthesized and decomposed. Among the latter, an important factor is the pH of the blood which is especially to be considered in the states of acidosis discussed in the present paper. In blood incubated in vitro, acidification to pH 7.0 to 7.3 leads to a rapid liberation of inorganic phosphorus at the expense of diphosphoglycerate, while reactions above pH 7.3 favor the esterification of inorganic phosphorus and synthesis of diphosphoglycerate.²¹ The fact that decomposition of diphosphoglycerate is favored by a range of reactions seen frequently in the blood in vivo offers an explanation

of one mechanism by which its concentration in the blood cells is reduced in states of acidosis such as those cited in Tables I, II, and III. Closely related to the effect of pH is the distribution of chloride between cells and serum. Observations of reciprocal relationships between changes in concentrations of chloride and diphosphoglycerate in the blood cells in a variety of conditions suggest that the concentration of chloride in itself may exert an influence on the synthesis and decomposition of diphosphoglycerate.

Other phosphorus stores of the body are, however, more important than the diphosphoglycerate of the blood as a source of the increased urinary phosphorus in acidosis. The fact that during the development of acidosis the increased phosphaturia at first is not accompanied by much, if any, increase in calcium excretion indicates that the phosphorus of soft tissues is liberated before that of bone. Phosphorus compounds of the soft tissues are closely related to those of the blood, and apparently are similarly affected by acidotic conditions. A rapidly developing severe acidosis may lead to exhaustion of the labile phosphorus reserves before the large phosphorus stores in bone can be mobilized. This loss in turn may lead to a series of interrelated disturbances. Since phosphorus is known to play an essential role in respiration and in carbohydrate metabolism, exhaustion of the labile phosphorus reserves can be expected to affect adversely the metabolic activity of various tissues. Added to this is the fact that the excretion of phosphates and other acid metabolites entails a loss of base which, if continued beyond a relatively short time, results in a lowered concentration of total base in the blood and tissues. The base deficit thus produced is a well-recognized factor in the dehydration of acidosis, and both base deficit and dehydration may aggravate the state of acidosis. Thus, acidosis from any cause is likely to create a vicious cycle in which different changes mutually intensify the severity of each other.

Such a vicious cycle can be visualized readily in the development of acidosis with gastroenteritis in infants. Losses of base in diarrheal stools result in base deficits in the blood and tissues, acidosis, and dehydration. Acidosis favors the decomposition of the labile organic acid-soluble phosphorus compounds of the blood and tissues, giving rise to increased phosphaturia; with this, losses of base in the urine augment those already occurring in the stools. The decomposition of diphosphoglycerate proceeds simultaneously with increases in concentration of chloride in the blood cells, shifts in distribution of chloride between serum and cells, and changes of pH, so that each set of changes can be said to influence the other.

Failure of excretion of phosphates in the urine, due to acute suppression of renal function, is followed by changes of the organic acid-soluble phosphorus and chloride in the blood cells in directions opposite to those described as occurring in chloride and diabetic acidosis in subjects with adequate renal function. Some of the effects of abrupt cessation of renal function have been illustrated in another paper in a chart representing blood changes in a dog after bilateral nephrectomy.^{7*} Briefly, those changes were progressive increase of nonprotein nitrogen and inorganic phosphorus in the blood; increase of organic acid-soluble phosphorus in the cells, accounted for by equal increases in the diphospho-

*Chart 3.

glycerate fraction; decrease of HCO_3 and Cl in both serum and cells. Such changes are typical of those found in the blood of many nephritic patients suffering from acute suppression of urinary excretion. However, not all cases commonly called "nephritic acidosis" show blood changes falling into this pattern.

The values listed in columns 1 and 2 of Table IV are, in fact, very much like those found at different times after nephrectomy in the experiment just described. In these two bloods, *high* concentrations of organic acid-soluble phosphorus in the cells accompanied low concentrations of carbon dioxide in the serum, and in the second, the chloride was low in both serum and cells. In column 3, however, is shown a picture fairly similar to those of other types of acidosis previously discussed, i.e., *low* concentration of organic acid-soluble phosphorus in the cells, with low carbon dioxide content of the serum and a high $\text{Cl}_c:\text{Cl}_s$ ratio. In column 4 a *normal* concentration of organic acid-soluble phosphorus accompanies decreased values for both carbon dioxide and pH in the serum, and a high $\text{Cl}_c:\text{Cl}_s$ ratio. These values probably resulted from a balance between the effects of acidosis and of phosphate retention, respectively favoring decreases and increases of organic acid-soluble phosphorus in the cells. Differences between the response to acidosis in these two patients, represented in columns 4 and 5, may have been due to slight differences in their ability to get rid of endogenous phosphates, and possibly also to differences in their phosphorus reserves, exhausted more or less during a long period of borderline acidosis. In terminal stages, "uremia" is characterized by great increases of organic acid-soluble phosphorus in the cells, with extreme acidosis; under such conditions the inorganic phosphorus is always very high. Presumably the increase of diphosphoglycerate in the cells under such conditions is due to the retention of phosphates, but the mechanism by which this increase occurs, together with concomitant shifts in other electrolytes, is by no means clear.

SUMMARY

The concentration of organic acid-soluble phosphorus in the blood cells is greatly reduced during severe acidosis in subjects with adequate renal function, following the ingestion of ammonium chloride, in diabetic coma, and in infants with profuse diarrhea. The decrease is in the diphosphoglycerate fraction, which appears to be one source of the increased phosphaturia that occurs in these conditions. High, normal, or low concentrations of organic acid-soluble phosphorus in the blood cells may be found during acidosis in nephritic subjects with low renal function, depending upon different conditions present in individual cases. The changes of phosphorus distribution are closely related to the state of other electrolytes in the blood, and they appear to constitute a valuable index of the functional state of the labile phosphorus reserves of the body.

REFERENCES

1. Forbes, E. B., and Keith, M. H.: A Review of the Literature of Phosphorus Compounds in Animal Metabolism, Wooster, Ohio, Technical Series, Bull. 5, 1914, p. 510.
2. Fitz, R., Alsberg, C. L., and Henderson, L. J.: Concerning the Excretion of Phosphoric Acid During Experimental Acidosis in Rabbits, *Am. J. Physiol.* 18: 113, 1907.
3. Henderson, L. J.: A Critical Study of the Process of Acid Excretion, *J. Biol. Chem.* 9: 403, 1911.

4. Haldane, J. B. S., Wigglesworth, V. B., and Woodrow, C. E.: The Effect of Reaction Changes on Human Inorganic Metabolism, *Proc. Roy. Soc., London*, s. B. 96: 1, 1924-25.
5. Byrom, F. B.: Blood Phosphorus in Health and Disease. IV. Phosphorus Content of the Blood in Diabetes Mellitus, *Brit. J. Exper. Path.* 10: 10, 1929.
6. Rapoport, S.: Ueber Phosphoglycerinsäure als Transportsubstanz des Blutphosphors und ihr Verhalten bei Experimenteller Ammonchloridazidose, *Biochem. Ztschr.* 289: 411, 416, 1937.
7. Guest, G. M., and Rapoport, S.: Role of Acid-Soluble Phosphorus Compounds in Red Blood Cells in Experimental Rickets, Renal Insufficiency, Pyloric Obstruction, Gastroenteritis, Ammonium Chloride Acidosis and Diabetic Acidosis, *Am. J. Dis. Child.* 58: 1072, 1939.
8. Marriott, W. McK., and Howland, J.: Phosphate Retention as a Factor in the Production of Acidosis in Nephritis, *Arch. Int. Med.* 18: 708, 1916.
9. Byrom, F. B., and Kay, H. D.: Blood Phosphorus in Health and Disease. II. Changes in the Phosphorus Compounds of the Blood in Renal Disease. *Brit. J. Exper. Path.* 8: 429, 1927.
10. Ashley, A., and Guest, G. M.: Distribution of Blood Phosphorus After Suppression of Renal Function, *J. Clin. Investigation* 13: 219, 1934.
11. Guest, G. M., and Rapoport, S.: Studies of Diphosphoglyceric Acid in Blood Cells, *Proc. Am. Soc. Biol. Chem. J. Biol. Chem.* 123: xlvii, 1938.
12. Rapoport, S., and Guest, G. M.: Role of Diphosphoglyceric Acid in the Electrolyte Equilibrium of Blood Cells: Studies of Pyloric Obstruction in Dogs, *J. Biol. Chem.* 131: 675, 1939.
13. Chabanier, H., and Lobo-Onell, C.: Exploration fonctionnelle des reins, Paris, 1930, Masson et Cie., Acidose et urémie, p. 505.
14. Chabanier, H., Guillaumin, C. O., Laudat, M., Lévy, M., Paget, M., and Vaille, C.: A propos du chlore sanguin, *Bull. Soc. chim. biol.* 19: 800, 1937.
15. Lévy, M.: L'acidose et l'insuffisance rénale aiguë chez le nourrisson, Paris, 1932, Masson et Cie.
16. Ribadeau-Dumas, L., Lévy, M., and Mignon, S.: Études sur les chlorémies plasmatique et globulaire du nourrisson, *Rev. franç. de pédiat.* 13: 1, 1937.
17. Gamble, J. L.: Extracellular Fluid, *Bull. Johns Hopkins Hosp.* 61: 151, 1937.
18. Hamilton, B., Kajdi, L., and Meeker, D.: The Acidosis of Acute Diarrhea in Infancy, *Am. J. Dis. Child.* 38: 314, 1929.
19. Guest, G. M., and Andrus, W. De W.: Chemical Studies of the Blood in High Intestinal Obstruction. I. Distribution of Phosphorus and Intracellular Changes, *J. Clin. Investigation* 11: 455, 1932.
20. Guest, G. M., and Rapoport, S.: Role of Diphosphoglycerate and Other Organic Acid-Soluble Phosphorus Compounds of the Red Blood Cells in the Electrolyte Equilibrium of the Blood, *Am. Assoc. for Advancement of Science, Symposium, Blood, Heart, and Circulation*, Washington, D. C., 1940, Science Press, p. 55.
21. Rapoport, S., and Guest, G. M.: Decomposition of Diphosphoglycerate in Acidified Blood: Its Relationship to Reactions of the Glycolytic Cycle, *J. Biol. Chem.* 129: 781, 1939.

SOME CHEMICAL OBSERVATIONS ON THE HUMAN HEART IN HEALTH AND DISEASE*

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DURING recent years the problem of myocardial failure has been attacked vigorously from many directions. Not least among these has been the chemical approach to the problem. As the new concept of the chemical nature of the mechanism of muscle contraction has been elaborated, new possibilities have arisen which, although at the present time in an embryonic state of development, may ultimately prove to be of great practical importance in clinical medicine.

It is not intended here to attempt to give a review of the entire field of the chemistry of the heart and of heart failure. Numerous reviews have appeared upon the subject, particularly those concerned with the gaseous metabolism of the heart and carbohydrate metabolism. Bodansky and Bodansky³ have recently summarized the chemical blood changes associated with cardiac insufficiency. The present communication will deal largely with a discussion of the chemical composition of the heart muscle itself and some of the factors that have been found to influence it.

NORMAL COMPOSITION

Heart muscle may be divided into an intracellular and extracellular phase, similar to that which has received much attention in skeletal muscle. It differs from skeletal muscle in that there is relatively more extracellular and less intracellular phase. Hastings, Blumgart, Lowry, and Gilligan¹⁵ have determined the normal phase relationships in the dog heart. They found that the water content of the intracellular phase paralleled that of other tissues which Hastings and his collaborators studied. The water content of the whole tissue is higher than that of skeletal muscle, but the extra water is associated with sodium and chloride and, therefore, assumed to be extracellular. In young dogs it was found that there was more extracellular fluid than in adult dogs. Samples from the left ventricle, right ventricle, and septum showed that the intracellular phase was the greatest in the left ventricle and least in the right ventricle.

In the human heart the intracellular phase is likewise smaller than in skeletal muscle. This is reflected in the lower concentration of potassium and the higher chloride and sodium content of the hearts examined after death by the Vanderbilt investigators,²⁰ and by other workers.²² The right ventricle of man contains considerably more extracellular phase than that of the dog,

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causing an apparent wide difference in the concentrations of potassium, creatine, phosphorus, and other substances between the two ventricles. However, there is no chemical evidence to indicate that this is due in any part to factors other than differences in the extracellular water, connective tissue, and fat content, all of which are greater in the right ventricle. We have found that the intracellular constituents, creatine, phosphorus, and potassium, are approximately equal in both ventricles when corrected to a constant water content in the collagen and fat-free tissue (unpublished observations).

CREATINE AND PHOSPHORUS COMPOUNDS

Since the discovery of phosphocreatine by Fiske and Subbarow¹³ in 1927, a number of investigators have directed special attention toward its role in the heart. Inasmuch as phosphocreatine cannot be determined in the human heart at autopsy, due to its extreme lability, studies upon its variations have been carried out either on animals experimentally or on the human heart by observing the creatine content. Creatine has proved to be a particularly easy substance to study, because, of all the compounds so far investigated, it has shown the greatest and probably the most significant variations. Due to its close association as phosphocreatine with the final steps in the transfer of energy from the chemical system to mechanical energy, and the ease with which it may be determined, it has lent itself to the study of many problems as an index of the net effect of any disease or experimental procedure upon the heart muscle.

Creatine is present in heart muscle in about half the concentration it attains in skeletal muscle. Phosphocreatine has been reported by various investigators as present in concentrations from one-tenth to one-third that of skeletal muscle. Its lower concentration in heart muscle than in skeletal muscle cannot be attributed to a difference in the amount of the intracellular phase, since there is a sharp decrease in the ratio of creatine to potassium. The distribution of phosphorus compounds is likewise greatly different from that of skeletal muscle. While the total phosphorus of the heart is about the same as that of skeletal muscle, a much greater part is present in the acid-insoluble fraction, most of which is phospholipid. The acid-soluble fraction is accordingly less, and this difference may be traced largely to the lower phosphocreatine content of the heart.

The chemical composition of the normal and failing human heart is shown in Table I. Where normal data were not available, average values for miscellaneous autopsy cases are given.

While the full implications of muscle creatine were not recognized until the discovery of phosphocreatine in 1927, and its subsequent correlation with muscle contraction, creatine has long been regarded as closely associated with muscle contraction.¹⁸ It is interesting to note that the chemical approach in the human myocardium to the problem of heart failure was first attacked through this compound and that to date no other substance has been conclusively shown to be as greatly affected by myocardial insufficiency. While much evidence has accumulated indicating that the loss of creatine is due to failure of the chemical system supplying energy for the resynthesis of phosphocreatine, creatine nevertheless remains as the outstanding substance by which the degree

TABLE I
THE CHEMICAL COMPOSITION OF THE HUMAN HEART
(MILLIGRAMS PER 100 GRAMS)

	NO. OF CASES	LEFT VENTRICLE	RIGHT VENTRICLE	REFERENCE
Water, Gm.				
Normal	5	78.9	79.2	Wilkins and Cullen ²⁰
Insufficiency	8	80.5	81.5	Wilkins and Cullen ²⁰
Chloride				
Miscellaneous	10	139	182	Muntwyler et al. ²⁵
Sodium				
Normal	5	92	107	Wilkins and Cullen ²⁰
Insufficiency	8	115	142	Wilkins and Cullen ²⁰
Potassium				
Normal	13	285	219	Mangun and Myers ²²
Normal	5	311	255	Wilkins and Cullen ²⁰
Insufficiency	8	258	200	Wilkins and Cullen ²⁰
Insufficiency	17	232	178	Mangun et al. ²³
Total phosphorus				
Normal	13	194	160	Mangun and Myers ²²
Normal	5	203	177	Wilkins and Cullen ²⁰
Insufficiency	8	170	149	Wilkins and Cullen ²⁰
Insufficiency	17	169	135	Mangun et al. ²³
Acid-soluble phosphorus				
Normal	36	89	-	Decherd and Blum ¹¹
Insufficiency	33	72	-	Decherd and Blum ¹¹
Creatine				
Normal (trauma)	13	203	165	Mangun and Myers ²²
Normal (trauma)	11	183	-	Herrmann et al. ¹⁷
Essentially normal	48	202	-	Cowan ⁸
Insufficiency	17	147	-	Cowan ⁸
Insufficiency	11	175	132	Linegar et al. ²⁰
Insufficiency	32	122	-	Herrmann et al. ¹⁷
Oxypurine nitrogen				
Miscellaneous	18	36.9	22.5	Mangun and Myers ²¹
Insufficiency	6	30.0	22.4	Mangun and Myers ²¹

of deficiency of the chemical mechanism may be evaluated in the human heart. This situation arises as a result of the close association of creatine with the final step in the transfer of energy to the mechanical phase of contraction and its stability and ease of measurement. Phosphocreatine and adenylypyrophosphate which are even more closely linked with contraction cannot be evaluated in the human heart because of their extreme lability.

Studies on the creatine content of the human heart began with the work of Constabel.⁷ In essentially normal persons he found values of 170 to 180 mg. Lower values were reported in persons whose heart action was poor, particularly where fatty changes in the myocardium were evident.

The greater part of the investigations on the human heart were undertaken after the discovery of phosphocreatine. For the sake of clarity, they will be discussed from the standpoint of interpretations placed upon them rather than chronologic order.

Normal Values.—The values reported by Constabel⁷ appear to be low in the light of more recent data. Vollmer²⁸ reported averages of 221 mg. and 173 mg.

of creatine for the left and right ventricles, respectively. In three normal persons, Bodansky² obtained values ranging from 220 mg. to 285 mg., presumably in the left ventricle. In 48 human hearts, which he considered to be normal, Cowan reported values ranging from 117 mg. to 264 mg., with an average of 194 mg. In 95 miscellaneous cases obtained within thirty-six hours post mortem, Linegar, Frost, and Myers²⁰ obtained an average of 208 mg. and 149 mg. for the left and right ventricles, respectively. Herrmann, Decherd, and Oliver²¹ reported an average of 175 mg. in 34 hearts which they considered to be essentially normal. In another series of miscellaneous cases, Mangun, Reichle, and Myers²² found average values of 199 mg. and 148 mg. in the left and right ventricles, respectively. Bodansky, Pileher, and Duff⁴ studied 212 males and 88 females and reported average values of 157 mg. and 163 mg., respectively, in the left ventricles of the two sexes.

To gain more accurate information about the normal creatine content of the human heart, Mangun and Myers²² obtained samples from a limited number of persons whose death was sudden. In 13 cases the creatine content of the left ventricle varied from 186 mg. to 218 mg., and averaged 203 mg., and the right ventricle varied from 154 mg. to 185 mg., and averaged 165 mg. These values were far more constant than the values found in any of our pathologic studies and indicate that the normal creatine content of the myocardium is fairly uniform, in contrast to variations of large magnitude in both directions in diseased persons.

EFFECT OF AGE, SEX, GENERAL CONDITION

Constabel⁷ was able to find no correlation between the creatine content of the myocardium and age and sex. Later studies, however, indicate that some correlation does exist. Seebof, Linegar, and Myers²¹ found that the creatine content of the newborn infants was approximately the same in both ventricles. The creatine content of both ventricles was found to rise rapidly and reach a maximum within the first year, after which there was no change up to the age of 30. Between the ages of 30 and 50 there appeared to be a slight drop, after which the values again increased. However, it is difficult to demonstrate any positive correlation during the later years because of the relatively small variations and the possibility of the incidence of disease at different age levels affecting the results. Bodansky, Pileher, and Duff⁴ recorded a slightly higher cardiac creatine content in women than in men.

A comparison of our normal group with miscellaneous pathologic cases indicates that the heart tends to maintain a high creatine level in the absence of severe cardiac involvement. Only rarely are values below 170 mg. encountered in the left ventricle in the absence of myocardial insufficiency or degenerative changes in the myocardium. We have encountered numerous cases in which the creatine content of the pectoralis major was sharply decreased without appreciable decrease in the myocardium. Occasionally the creatine content of the heart is low in acute infections, possibly due to a direct action of the bacteria or their toxic products on the myocardium. In chronic conditions, such as carcinoma and diabetes, cardiac creatine values tend to be slightly lower than normal, but seldom fall in the range encompassing most cases of myocardial

insufficiency. Because of the overlapping of pathologic conditions, it is difficult to convey the true status of individual cases in tabular form without accompanying diagnoses.

We have encountered numerous cases in our studies in which the myoeardial creatine values are elevated markedly without apparent cause. . Particularly prominent in this group are cases with histories of fever. Daus and Myers (unpublished data) have recently studied the effect of experimental hyperthermia on the creatine content of rat skeletal muscle. They found the creatine content increased, partly due to dehydration and partly due to an increased creatine content per unit of dry tissue. Whether this increase is due to a loss of muscle solids without a parallel loss of creatine, or whether there was an increased rate of formation could not be determined from the data obtained.

In uremia Linegar, Frost, and Myers²⁰ found a definite elevation of creatine in both heart and skeletal muscles. By the retention of creatinine, the creatine-creatinine equilibrium would be shifted toward the former. It is interesting to note that Mangun, Reichle, and Myers,²³ have also observed a tendency toward elevation of phosphorus and potassium, suggesting the possibility that the extra creatine may be retained as the potassium salt of creatine phosphoric acid. Whether this might exert a beneficial effect on the heart by increasing the energy and base reserve of the myocardium is an interesting speculation.

MYOCARDIAL INSUFFICIENCY

Since the first investigation of creatine in the human heart by Constabel,⁷ it has become more and more apparent that this substance is decreased in the failing heart. On this point, all investigators in this field are agreed. The work of Vollmer,²⁸ Cowan,⁸ Linegar, Frost, and Myers,²⁰ Bodansky and Pilcher,⁴ Herrmann and his associates,^{16, 17} and Mangun, Reichle and Myers,²³ have clearly demonstrated that a loss of creatine is usually associated with myocardial decompensation. The underlying cause of the decrease in creatine content of the myocardium, however, has often been challenged. It has been suggested at various times that the decrease in creatine was due to anatomic changes in the muscle, to edema, to injury of the cell membranes, permitting its diffusion out of the cells, and to hypertrophy of noncreatine containing parts of the cell. The final alternative is that the loss of creatine is due to chemical events within the cell, an inability to maintain the creatine in the nondiffusible form of phosphocreatine.

Let us examine more closely the changes that take place in the failing heart. Determinations of the water content by various investigators have demonstrated that only a small fraction of the drop in creatine content can be assigned to this factor.⁹ While there is usually a small increase in the water content of the failing heart, and occasionally a significant increase,^{24, 29} this factor is negligible with respect to creatine, although it may be of importance in itself as a factor interfering with the metabolic activity of the myocardium. The possibility that anatomic alterations, such as fatty infiltration and increases in connective tissue, may be responsible have been discounted by workers who have examined the heart histologically in conjunction with their analyses.^{8, 16} In our series of studies we have determined the water, fat, and collagen content in a limited num-

of creatine for the left and right ventricles, respectively. In three normal persons, Bodansky² obtained values ranging from 220 mg. to 285 mg., presumably in the left ventricle. In 48 human hearts, which he considered to be normal, Cowan reported values ranging from 117 mg. to 264 mg., with an average of 194 mg. In 95 miscellaneous cases obtained within thirty-six hours post mortem, Linegar, Frost, and Myers²⁰ obtained an average of 208 mg. and 149 mg. for the left and right ventricles, respectively. Herrmann, Decherd, and Oliver²¹ reported an average of 175 mg. in 34 hearts which they considered to be essentially normal. In another series of miscellaneous cases, Mangun, Reichle, and Myers²³ found average values of 199 mg. and 148 mg. in the left and right ventricles, respectively. Bodansky, Pileher, and Duff⁴ studied 212 males and 88 females and reported average values of 157 mg. and 163 mg., respectively, in the left ventricles of the two sexes.

To gain more accurate information about the normal creatine content of the human heart, Mangun and Myers²² obtained samples from a limited number of persons whose death was sudden. In 13 cases the creatine content of the left ventricle varied from 186 mg. to 218 mg., and averaged 203 mg., and the right ventricle varied from 154 mg. to 185 mg., and averaged 165 mg. These values were far more constant than the values found in any of our pathologic studies and indicate that the normal creatine content of the myocardium is fairly uniform, in contrast to variations of large magnitude in both directions in diseased persons.

EFFECT OF AGE, SEX, GENERAL CONDITION

Constabel⁷ was able to find no correlation between the creatine content of the myocardium and age and sex. Later studies, however, indicate that some correlation does exist. Seebof, Linegar, and Myers²⁷ found that the creatine content of the newborn infants was approximately the same in both ventricles. The creatine content of both ventricles was found to rise rapidly and reach a maximum within the first year, after which there was no change up to the age of 30. Between the ages of 30 and 50 there appeared to be a slight drop, after which the values again increased. However, it is difficult to demonstrate any positive correlation during the later years because of the relatively small variations and the possibility of the incidence of disease at different age levels affecting the results. Bodansky, Pileher, and Duff⁴ recorded a slightly higher cardiac creatine content in women than in men.

A comparison of our normal group with miscellaneous pathologic cases indicates that the heart tends to maintain a high creatine level in the absence of severe cardiac involvement. Only rarely are values below 170 mg. encountered in the left ventricle in the absence of myocardial insufficiency or degenerative changes in the myocardium. We have encountered numerous cases in which the creatine content of the pectoralis major was sharply decreased without appreciable decrease in the myocardium. Occasionally the creatine content of the heart is low in acute infections, possibly due to a direct action of the bacteria or their toxic products on the myocardium. In chronic conditions, such as carcinoma and diabetes, cardiac creatine values tend to be slightly lower than normal, but seldom fall in the range encompassing most cases of myocardial

insufficiency. Because of the overlapping of pathologic conditions, it is difficult to convey the true status of individual cases in tabular form without accompanying diagnoses.

We have encountered numerous cases in our studies in which the myoeardial creatine values are elevated markedly without apparent cause. Particularly prominent in this group are cases with histories of fever. Daus and Myers (unpublished data) have recently studied the effect of experimental hyperthermia on the creatine content of rat skeletal muscle. They found the creatine content increased, partly due to dehydration and partly due to an increased creatine content per unit of dry tissue. Whether this increase is due to a loss of muscle solids without a parallel loss of creatine, or whether there was an increased rate of formation could not be determined from the data obtained.

In uremia Linegar, Frost, and Myers²⁰ found a definite elevation of creatine in both heart and skeletal muscles. By the retention of creatinine, the creatine-creatinine equilibrium would be shifted toward the former. It is interesting to note that Mangun, Reiehle, and Myers,²³ have also observed a tendency toward elevation of phosphorus and potassium, suggesting the possibility that the extra creatine may be retained as the potassium salt of creatine phosphoric acid. Whether this might exert a beneficial effect on the heart by increasing the energy and base reserve of the myocardium is an interesting speculation.

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ber of cases and have been unable to demonstrate any serious alterations in the values for creatine, phosphorus, and potassium after correction for these factors. Occasionally in a heart the fat and collagen content, particularly of the right ventricle, have been found to be increased, but these alterations in individual cases exerted a negligible effect upon the group of cases studied in this manner. It would, nevertheless, be highly desirable to have more extensive studies conducted upon the collagen content of the heart, especially in relation to age, hypertrophy, and infarction.

The possibility that injury of the cell membrane is responsible for the loss in creatine seems hardly credible. There is ample evidence in the literature to indicate that creatine may be taken up by muscle as well as released by it. The more plausible explanation, then, would be that the creatine saturation level of the myocardium is decreased as a result of a decrease in phosphocreatine.

The studies of the Vanderbilt investigators²⁰ demonstrated that there is a loss of potassium and phosphorus from the heart in congestive heart failure. However, there appears to be some conflict of opinion as to the possible role of potassium as a contributory cause in heart failure. Herrmann and his co-workers¹⁶ concluded that the slight losses of potassium which they observed were not significant. It is true that the per cent loss of potassium from the failing heart is considerably less than that of creatine and more variable. The decrease of phosphorus in the failing heart has been confirmed by Mangun, Reichle and Myers²³ and by Decherd and Blum.¹¹ The latter authors demonstrated that the phosphorus loss was confined mostly to the acid-soluble fraction, an observation in full accord with some of our unpublished studies.

Work carried out in this laboratory led us to believe that a close correlation existed between the losses of creatine, phosphorus, and potassium. In analyzing data obtained on a series of autopsy cases, it soon became apparent that the loss of creatine was far greater on a percentage basis than that of either potassium or phosphorus. However, in further studying the results, Mangun, Reichle and Myers²³ noted that the loss in millimoles was more than twice as large for both potassium and phosphorus as for creatine. It was accordingly concluded that the observed changes were due to a loss of the dipotassium salt of creatine phosphoric acid, together with other acid-soluble phosphorus compounds.

In 1937 Burns and Cruickshank⁵ published a very interesting paper in which they studied the effects of asphyxia and fatigue on the excised heart of the cat. They found that when the heart was arrested in certain stages of asphyxia a loss of phosphagen (phosphocreatine) preceded that of adenylypyrophosphate, with a loss of about 80 and 60 per cent of phosphagen and adenylypyrophosphate, respectively, with complete asphyxiation. Similar results were obtained with the heart of the dog. In the presence of oxygen, however, fatigue produced only a slight loss of phosphagen, with a relatively greater loss of adenylypyrophosphate, about a 25 and 50 per cent loss of phosphagen and adenylypyrophosphate, respectively.

To determine more accurately what changes took place in the creatine and phosphorus compounds of the heart in insufficiency, Mangun and Roberts²²

studied the distribution of phosphorus compounds in the left ventricle of the dog's heart in late aortic insufficiency. No decrease in either phosphocreatine or adenylypyrophosphate was observed in dogs with varying degrees of hypertrophy without decompensation. However, in two dogs in the very late stages of aortic insufficiency with decompensation, it was found that there was a decrease in creatine, total acid-soluble phosphorus, phosphocreatine, and adenylypyrophosphate. The most striking change in the hearts of these dogs was the marked loss of adenylypyrophosphate, which was decreased about 40 per cent in both animals. The loss of phosphocreatine was less apparent, being slightly lower than the lowest normal value in a series of 8 normal animals and about 15 per cent below the average normal value. The loss of acid-soluble phosphorus was completely accounted for by the decreases in adenylypyrophosphate and phosphocreatine. The loss of creatine was about half accounted for by the decrease in phosphocreatine.

The findings in the animal experiments of Mangun and Roberts²⁴ have essentially fitted into our concept of the chemical nature of heart failure. In addition, their findings paralleled those of Burns and Cruickshank⁵ on isolated animal hearts fatigued in the presence of oxygen rather than their experiments on asphyxial arrest. The observations are too few to permit the drawing of definite conclusions as to the relative roles of fatigue and anoxemia in decompensation, but indicate that the former may be the predominant factor.

The strategic position occupied by adenylypyrophosphate with respect to phosphocreatine, glycolysis, energy transfer, and phosphorus transfer seemed to warrant further investigations. Since it is impossible to determine the concentration of adenylypyrophosphate in the human heart due to its rapid breakdown to adenylic acid and subsequent deamination, we attempted to determine it by measuring the oxypurine nitrogen content. This we did by treating a trichloroacetic acid extract of heart muscle with nitrous acid in a boiling water bath and subsequently determining the purine bases by the method of Hitchings (1933). A preliminary report of nine cases was given before the 1940 meeting of the American Society of Biological Chemists. Since that time a total of 24 cases have been studied. In 6 cases of myocardial insufficiency the average oxypurine content of the left ventricle was about 15 per cent lower than the average of the remaining 18 cases in the series. Only one of the 6 cases showed a relatively high oxypurine content; this was a case of acute rheumatic fever of short duration following childbirth. Total acid-soluble phosphorus and creatine determinations were also made on this series. In general, these substances varied in the same direction as the purine content. In the group with insufficiency the total acid-soluble phosphorus was about 15 per cent below that of the other cases and the creatine was about 33 per cent lower.

CARDIAC HYPERTROPHY

The question of the effect of cardiac hypertrophy upon the chemical composition of the human heart is of importance both as a phenomenon in itself and in relation to myocardial failure. Herrmann and his co-workers^{16, 17} and others look upon hypertrophy as intimately associated with failure, possibly the principal factor in bringing about an eventual inability of the metabolic trans-

fer between the blood and muscle to keep pace with the increased demands upon the energy output. Roberts, Wearn, and Badal²⁶ have shown that as a result of the increase in diameter of the muscle fibers there is a decrease in the number of capillaries per square millimeter of heart muscle. This leads to an increased radial distance the metabolites must diffuse from each capillary, a situation which may conceivably lead to decreased nutrition and anoxia of the muscle fibers.

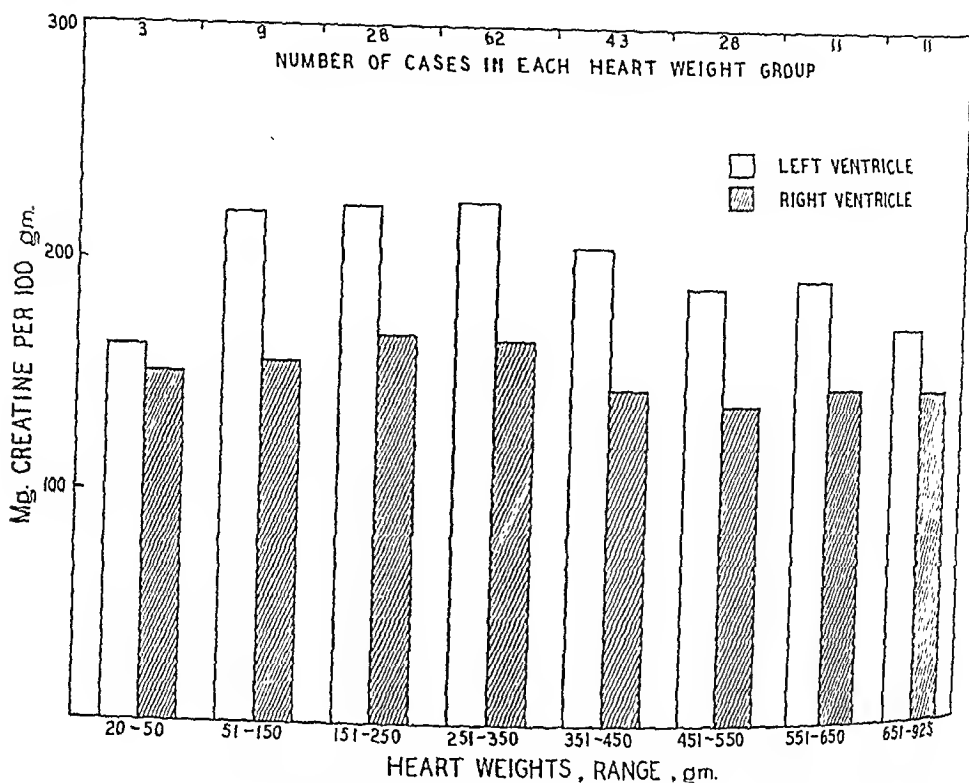


Fig. 1.—The relationship of heart weight to the creatine content of the left and right ventricles of the human heart in 195 autopsy cases.

It is difficult to obtain accurate information upon the effect of hypertrophy on the chemical composition of human heart muscle because of the variability of the findings and the influence of other factors. Cowan⁸ observed that in hearts with hypertrophy the average creatine concentration was slightly higher than in hearts without hypertrophy, but did not believe the slight difference to be significant.

In work carried out on human and animal hearts in this laboratory we have persistently noted a tendency toward higher creatine, phosphorus, and potassium values, with increases in heart weight. These differences, however, have been relatively small and of such magnitude as might be expected from variations in the amount of intracellular phase in the myocardium. The correlation between heart weight and chemical composition was most evident in cases of sudden death and in the hearts of dogs where the heart weight-body weight ratio was used as an index of hypertrophy (Mangun and Roberts, unpublished data). Most of these hearts, however, were within the range of what

is considered to be physiologic hypertrophy. To observe more closely the correlation between heart weight and creatine content throughout the normal and pathologic range, we have arranged the cases studied by Seecof and co-workers²⁷ and Mangun and associates^{22, 23} according to heart weights (Fig. 1). It is seen that the creatine content of both ventricles is low in hearts weighing less than 50 Gm. It rises rapidly, reaching a maximum in the left ventricle in the 51 to 150 Gm. hearts, and in the right ventricle in the 151-250 Gm. hearts it maintains its highest level up to 350 Gm., and then begins to fall, reaching its lowest level in adult hearts in the group weighing over 650 Gm. It should be pointed out that one should not lose sight of the effects of age and the incidence of myocardial insufficiency in these groups. The low heart weight group is composed of very young persons, less than a year old, and the incidence of heart failure increases progressively with each increment of heart weight. Relatively high creatine values are frequent throughout the range of heart weights, indicating that hypertrophy itself does not necessarily limit the saturation level of the muscle. Nevertheless, the fact remains that there is a statistical correlation between cardiac creatine content and heart weight.

SIGNIFICANCE OF THE CHEMICAL CHANGES IN HEART FAILURE

It has often been stated by clinicians that the observed changes in heart muscle in congestive failure were most likely due to anatomic changes in the muscle and, therefore, possessed no significance beyond a reflection of these anatomic changes. However, recent studies on electrolytes and water, connective tissue, fat, and other constituents of the heart indicate most clearly that the pronounced loss of creatine in myocardial failure cannot be explained upon that basis. There is a much greater per cent decrease in the concentration of creatine than can be explained upon any basis, except that part of the creatine has been lost from cells that are still retaining their identity insofar as the electrolyte pattern is concerned. In our experience, we are led to believe that a considerable part of the electrolyte changes occurring in failing hearts may be due to the intracellular disturbances in the phosphorus compounds, notably phosphocreatine and adenylypyrophosphate, with a concomitant extracellular edema or possibly a diffusion of extracellular electrolytes and water into the cells. As pointed out by Hastings and his collaborators,¹⁵ the present evidence permits either interpretation of the observed facts.

There is now evidence to indicate that simple cardiac hypertrophy is not responsible for the chemical changes observed in failing hearts. On the contrary, there appears to be an increase in the creatine content of the heart, and of other intracellular constituents, at least until a marked degree of hypertrophy has been attained. These changes may be due largely to an increase in the intracellular phase without a parallel increase in extracellular phase.

It should be stressed that the chemical events which we have observed in myocardial failure and experimental aortic insufficiency are those which have also been found to occur experimentally in cardiac and voluntary muscle as a result of anoxemia and fatigue. These aspects of chemical heart failure have been discussed in considerable detail by Herrmann and his co-workers. The role of hypertrophy in effecting a possible deleterious influence on the blood

supply and gaseous and nutritional exchange of the heart has been the subject of numerous experimental studies by Roberts, Wearn, and Badal,²⁶ Herrmann and collaborators,¹⁶ and others.

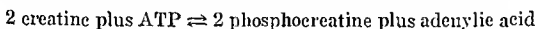
At the present time there appears to be no specific application of our knowledge of the chemistry of the heart which can be applied to the treatment of myocardial insufficiency. Nevertheless, there is a definite relationship between the chemical events in the heart and the therapy applied to congestive failure. Let us visualize the chemical system in the heart as a series of reactions designed to transfer energy from the substances carried to the muscle into mechanical energy of contraction. The ability of the heart at any given time to contract will depend upon the summated result of all the factors that contribute this energy versus all those factors which tend to dissipate, consume, or interfere with the production. So far as we know at the present time, phosphocreatine and adenylypyrophosphate are the two substances most closely associated with the final stage in the transfer of chemical energy to mechanical energy. To build up this final reaction to its highest level, the internist is, therefore, interested in accomplishing two objectives: increasing the rate at which energy is made available and decreasing the demands upon the system. In other words, we are interested in maintaining the optimum concentration of phosphocreatine and adenylypyrophosphate and yet having it supplying energy at an adequate rate, with the least possible dissipation. In the compensated heart the relative rates of the reactions centering about the phosphocreatine-adenylypyrophosphate system are such that energy input and energy expenditure are in equilibrium at a point where adequate amounts of phosphocreatine and adenylypyrophosphate are present. It is the task of the internist to maintain or improve the physiologic conditions which preserve this equilibrium.

At the present time most of the established methods of treating heart failure are probably effective largely because they decrease the demands upon the heart, thus permitting the energy input to keep abreast of the expenditure. The most outstanding of these types of therapy are rest, digitalis, and the removal of edema through regulation of the water and electrolyte balance. The latter may exert some direct beneficial effect upon the myocardium by removing edema fluid from that tissue, thereby accelerating the metabolic transfer. The possible direct action of digitalis upon the heart muscle still remains a subject of controversy. However, by overcoming tachycardia and irregularities of the heart it may decrease the expenditure of energy and increase the efficiency, a highly desirable effect from a chemical viewpoint.

While it is generally believed that the fundamental causes of decompensation may be attributed to increased demands upon the heart to perform work and decreased ability to perform such work as a result of disturbances in the coronary circulation, these considerations should by no means limit the possibilities of bolstering the heart action by measures directly affecting the myocardium. Attempts have been made to increase the creatine content of the heart experimentally or to prevent its loss by its addition to perfusing fluids, but these measures have met with little or no success. Similar attempts to increase the intracellular electrolytes of patients by feeding dipotassium phosphate have likewise met with failure.¹⁰ Decherd and Herrmann¹² have studied

the effect of various amino acids on the creatine content of the perfused rabbit heart. Only *DL*-alanine appeared to have an effect, and it seemed to act as a creatine-sparing substance. Glycine, glutamic acid, arginine, aspartic acid, methyl guanidine, and creatine had no such effect. Kalter¹⁹ administered glycine to patients with heart disease and reported a number of cases in which it apparently exerted a beneficial effect. Herrmann and Decherd¹⁶ have also reported some cases in which the administration of glycine appeared to have encouraging effects. Such studies are difficult to control, and the interpretations should be very cautious, due to the variability of such patients even without therapy.

The experimental finding that adenylypyrophosphate suffers a severe loss in anoxemia and fatigue,⁵ and in late aortic insufficiency,^{2*} and that there is a decrease in the extractive purine content of the human heart in myocardial failure²¹ suggests that disturbances in the metabolism of this compound may be of considerable significance. According to Lohmann's reaction:



phosphocreatine and adenylypyrophosphate exist in muscle in equilibrium with one another, the phosphocreatine acting as a donor of phosphate to maintain the nucleotide in a full state of phosphorylation at times when the transfer of phosphate from phosphopyruvic acid is inadequate. In the failing heart this mechanism may not suffice and there would be not only the depletion of the two compounds, but also the probability that a considerable part of the free adenylic acid would be deaminized to inosinic acid and thus removed from the system until reamination could be effected. It would appear that this problem should be investigated, and if this deamination does occur, it might prove of great value to learn more about the mechanism of reamination of inosinic acid to adenylic acid, and the effect of amino acids and other compounds upon this reamination. At present this is purely speculative and must await experimental investigation.

Finally let us emphasize that a decompensated heart, though it may be rendered capable of performing a limited amount of work sufficient to maintain the patient in a state of rest, cannot be considered to have recovered, from a chemical point of view, until a complete restitution of the chemical mechanism has been effected. Chemical deficiencies of the myocardium associated with, or resulting from, myocardial insufficiency must certainly constitute limiting factors in the process of recovery.

The chemical substances discussed in this paper by no means encompass the whole realm of possibilities. The number and variety of compounds concerned with the metabolism of the heart are constantly expanding, and new facts are being uncovered which throw light upon the question of how carbohydrates, fats, and proteins, their intermediate products, both in aerobic and anaerobic conditions, supply the energy of contraction. It is indeed possible that somewhere along the way we shall uncover information leading to a direct attack upon the complex problem of myocardial failure.

REFERENCES

1. Alburn, H. E., and Myers, V. C.: The Creatine, Phosphorus, and Collagen Content of Different Sections of the Dog Heart, *J. Biol. Chem.* 131: 713, 1939.
2. Bodansky, M.: Creatine in Human Muscle, *J. Biol. Chem.* 91: 147, 1931.

3. Bodansky, M., and Bodansky, O.: *Biochemistry of Disease*, New York, 1940, Macmillan Co.
4. Bodansky, M., Pilcher, J. F., and Duff, V. B.: The Clinical Significance of the Creatine Reserve of the Heart, *Arch. Int. Med.* 59: 232, 1937.
5. Burns, W., and Cruickshank, W. E. H.: Changes in Creatine, Phosphagen and Adenylpyrophosphate in Relation to the Gaseous Metabolism of the Heart, *J. Physiol.* 91: 314, 1937.
6. Calhoun, J. A., Cullen, G. E., Clarke, G. H., Harrison, T. R.: Studies in Congestive Heart Failure. VIII. The Effect of the Administration of Dibasic Potassium Phosphate on the Potassium Content of Certain Tissues, *J. Clin. Investigation* 9: 693, 1931.
7. Constabel, F.: Über den Kreatingehalt des menschlichen Herzmuskels bei verschiedenen Krankheitszuständen, *Biochem. Ztschr.* 122: 152, 1921.
8. Cowan, D. W.: The Creatine Content of the Myocardium of Normal and of Abnormal Hearts, *Am. Heart J.* 9: 378, 1934.
9. Cowan, D. W.: The Creatine Content and the Weight of the Ventricles in Experimental Hyperthyroidism and After Thyroparathyroidectomy, *Am. J. Physiol.* 109: 312, 1934.
10. Cullen, G. E., and Wilkins, W. E.: Electrolytes in Human Tissues. I. The Digestion of Tissue and Other Biological Material and the Subsequent Determination of Various Electrolytes, *J. Biol. Chem.* 102: 403, 1933.
11. Decherd, G. M., and Blum, J. E.: Phosphorus Fractions in Human Heart Muscle, *Proc. Soc. Exper. Biol. & Med.* 38: 341, 1938.
12. Decherd, G., and Herrmann, G.: Effect of Amino-Acids on the Creatine Content of Rabbit Heart Muscle, *J. Clin. Investigation* 14: 706, 1935.
13. Fiske, C. H., and Subbarow, Y.: Nature of "Inorganic" Phosphorus in Voluntary Muscle, *Science* 65: 401, 1927.
14. Gross, Harry: Water Content of the Myocardium in Hypertrophy and Chronic Congestive Failure, *J. LAB. & CLIN. MED.* 25: 899, 1940.
15. Hastings, A. B., Blumgart, H. L., Lowry, O. H., and Gilligan, D. R.: Chemical Changes in the Heart Following Experimental Temporary Coronary Occlusion, *Tr. A. Am. Physicians* 54: 237, 1939.
16. Herrmann, G., and Decherd, G. M., Jr.: Chemical Nature of Heart Failure, *Ann. Int. Med.* 12: 1233, 1939.
17. Herrmann, G., Decherd, G., and Oliver, T.: Creatine Changes in Heart Muscle Under Various Clinical Conditions, *Am. Heart J.* 12: 689, 1936.
18. Hunter, A.: Creatine and Creatinine. Monographs on Biochemistry, New York, 1923, Longmans, Green & Co.
19. Kalter, S.: Ueber Glykokallbehandlung degenerativedystrophischer Herzmuskelkrankungen, *Deutsche med. Wchnschr.* 62: 1936.
20. Linegar, C. R., Frost, T. T., and Myers, V. C.: Variation in Creatine Content of Human Cardiac and Voluntary Muscle at Autopsy, *Arch. Int. Med.* 61: 430, 1938.
21. Mangun, G. H., and Myers, V. C.: Purine Content of Human Cardiac and Voluntary Muscle, *J. Biol. Chem. Proc.* 133: proc. lxii, 1940.
22. Mangun, G. H., and Myers, V. C.: Normal Creatine, Phosphorus and Potassium Content of Human Cardiac and Voluntary Muscle, *J. Biol. Chem.* 135: 411, 1940.
23. Mangun, G. H., Reichle, H. S., and Myers, V. C.: Possible Implications of Variations in Creatine, Phosphorus and Potassium Content of Human Cardiac and Voluntary Muscle, *Arch. Int. Med.* In press.
24. Mangun, G. H., and Roberts, J. T.: Phosphorus Compounds in the Dog Heart, *Proc. Soc. Exper. Biol. & Med.* In press.
25. Muntwyler, E., Hanzal, R. F., Mangun, G. H., and Way, C. T.: The Electrolyte Content of Human Tissue, *Proc. Soc. Exper. Biol. & Med.* 35: 555, 1936.
26. Roberts, J. T., Wearn, J. T., and Badal, J. J.: The Capillary-Muscle Ratio in Normal and Hypertrophied Human Hearts, *Proc. Soc. Exper. Biol. & Med.* 38: 322, 1938.
27. Seecof, D. P., Linegar, C. R., and Myers, V. C.: The Difference in Creatine Concentration of the Left and Right Ventricular Cardiac Muscle, *Arch. Int. Med.* 53: 574, 1934.
28. Vollmer, H. Z.: Untersuchungen über den Kreatin- und Phosphorsäuregehalt verschiedene Herzteile, *Ztschr. f. d. ges. exper. Med.* 65: 522, 1929.
29. Wilkins, W. E., and Cullen, G. E.: Electrolytes in Human Tissue. III. A Comparison of Normal Hearts With Hearts Showing Congestive Heart Failure, *J. Clin. Investigation* 12: 1063, 1933.

KIDNEY PARENCHYMA, CIRCULATING BLOOD PLASMA, AND TISSUE FLUIDS IN DIURESIS*

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THERE has been an increasing interest lately in the problems of the appearance of edema and of its dissipation by therapeutic measures. The development of new agents and of combinations of diuretics based on modern conceptions of different points and modes of action of the various types of drugs has been responsible for much progress in this field. On the European continent an agent is considered as a diuretic only when it increases the urinary output to the extent that there is a gross decrease in the body weight. Under the influence of Addis,¹ in this country we are content to speak of a diuresis when, as a result of the exhibition of a given therapeutic measure, there is a rise in the urinary output from less than 1 c.c. to 3 c.c. or more per minute, an increase of at least 100 per cent. The urinary output rises sometimes as high as 5 to 10 c.c. per minute at the height of the drug effect. However, a full explanation of exactly how diuresis is produced is not yet forthcoming. Nollenbruch² has discussed exhaustively a great number of the theories of water metabolism and diuresis, certainly many of which are still quite abstruse. A tremendous amount of research, experimental and clinical investigation, has been vigorously pursued in these fields in many attempts to establish the mode of action of diuretics.

Our clinical studies³ prosecuted during the past decade with mercurial and xanthine diuretics and combinations of the two drugs have yielded some interesting theoretical as well as practical information. The comparison of diuretics is a simple subject about which much has been written, while the theory of diuresis is complex and justifies further discussion. Clinicians must not lose sight of the fact that a great volume of work has been done in each chapter and each subchapter of water and salt exchange. The phases touched upon have been numerous. Studies have encompassed water intoxication; the extrarenal storage and relinquishment of fluids and electrolytes; the surrender of water by the interstitial tissues; the role of the tissues; the role of the liver; the water and salt balance; the water passage through the tissues; the genesis and significance of tissue edema; water retention and diuresis effected by sodium chloride; the plasma volume, electrolyte concentration; albumin and globulin levels and ratios and colloid osmotic pressure and viscosity; possible effects of water, mercurials, and xanthines on electrolyte and plasma protein balances; the role of the capillary walls and intracapillary blood pressure; tissue fluid pressures versus colloidal osmotic pressure; the circulation and kidney function; systemic blood, venous, capil-

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lary, and glomerular pressures and blood flow as factors in diuresis; the blood constituents in relation to renal function; the kidneys functioning as the excretory organ. Many other questions have been studied. Literally hundreds of investigators have attacked these problems and sought to elucidate the complicated mechanisms. Unfortunately, much of the conflicting older experimental data had been obtained from studies on rabbits. Rabbits have been known to be notoriously susceptible to confusing nervous shifts in physiologic processes. Most of the diuretic experimental work must, therefore, be verified on large dogs before it can be accepted.

ANATOMICAL KIDNEY UNIT AND ITS PHYSIOLOGIC RELATIONSHIPS³⁻⁷

Into the uppermost end of each of one and one-half millions plus nephron tubules, afferent arterioles coil into an expansive network, invaginating the delicate epithelial layer of Bowman's capsule forming the broad filtration surface of the glomerular tuft. The surface area of the glomerular capillary bed is calculated to be about 1,000,000 square millimeters. Of similar importance is the anatomic fact that the efferent arteriole from each glomerulus follows the renal unit pattern and breaks up into a capillary bed in intimate contact with the continuation of the nephron, the epithelial-lined proximal and distal convoluted tubules. Some few direct vessels to the tubular plexuses have been found by MacNider to be present in diseased kidneys. Through these units the blood is brought into contact with the epithelial cells of a semipermeable membrane. The cardiovascular renal system tends to maintain a remarkable constancy in the general bodily milieu or homeostasis. Some water and some salts are eliminated to maintain the osmotic balance and the acid-base equilibrium, and the nitrogenous waste products are removed from the blood and discharged from the body. Glomerular filtration and active tubular reabsorption are now generally considered to be most significant factors in the maintenance of normal water and salt exchange. A consideration of the anatomy, and particularly of the blood supply of the nephron, makes it obvious that when the glomerulus fails in function, the tubule follows suit closely.

THEORIES OF KIDNEY FUNCTION^{4-6, 9-11}

The theories of kidney function have undergone changes since 1842 when Bowman postulated that water was secreted by the glomerular tuft and that the uriniferous products were secreted by the tubular epithelium, added to the water, and collected by the ducts as the urine. Ludwig in 1844 contended that production of urine consisted in the mechanical filtration of a protein-free solute through the glomerular membrane and reabsorption by diffusion of some salts and water. In 1874 Heidenhain revived the vitalistic secretion theory, adding that in addition to water, chlorides were selected and secreted by the glomerular epithelium, while organic nitrogenous waste substances and dyes were secreted by the lining cells of the tubules.

Barcroft and Straub¹⁰ in 1910 replaced blood plasma with Ringer's solution, retaining the red blood cells to insure oxygenation, and perfused this through intact kidneys with the production of an augmented urine flow without a rise

in blood pressure. Kuowilton in 1911 found that the addition of a colloid as gum acacia to the plasmapheresed erythrocyte solution or to a hypertonic glucose or saline solution introduced intravenously prevented a diuresis. Cohnheim in 1912 demonstrated that glomerular membrane cells had no selective secretory function, for if the body was deprived of salt, sodium chloride still appeared in the glomerular filtrate and had to be conserved by tubular reabsorption.

Cushny in 1917 conceived of a purely physical glomerular ultrafiltration and a nonvital selective diffusion tubular reabsorption. The plasma protein-free glomerular filtrate was postulated to be identical in electrolyte composition with plasma, contained water, salts, particularly sodium chloride, and also urea, uric acids, amino acids, creatinine, creatine, and glucose. While the tubular epithelium permitted diffusion through it of those threshold substances useful in the body economy and in maintaining water balance, there were reclaimed by tubular reabsorption all of the glucose, amino acids, and some of the salts and water. Cushny vehemently opposed every suggestion of tubular excretion.

Starling, as early as 1899, demonstrated the osmotic pressure of blood colloids to be 30 mm. Hg by preparing a filtrate of blood plasma through a gelatin filter, and with Verney in 1924, he added to Cushny's conception the theory of tubular secretion of dyes and urea, later confirmed by Hober and Marshall in 1930. Richards between 1910 and 1924, and his students between 1924 and 1938, by capsular and tubular microcatheterization proved the glomerular filtrate theory of Cushny's to hold and the actual specific reabsorption of various substances by the proximal and others by the distal convoluted tubules.

Rehberg in 1926 agreed to the glomerular filtration of a dilute dialysate of the blood plasma, but he too would endow the tubular epithelium with power of discrimination in reabsorption. He considered a substance threshold only if the percentage in reabsorbed fluid, under certain conditions, was higher than in the blood. Creatinine alone he accepted as entirely a waste totally nonthreshold substance, with phosphates, calcium, and sulfates as possibly so.

Homer W. Smith and associates, particularly Shannon in 1935, demonstrated the fact that creatinine was not an absolute index of glomerular filtration, but was apparently added by the tubular epithelial cells. In addition, urea, dyes as phenol red, and organic iodine preparations as hippuran, diodrast, skiodan, opax have been proved to be added by tubular excretion. D-xylose, raffinose, sucrose, and inulin were found to be the best indicators of glomerular filtration. The current theory generally accepted considers the glomerular fluid to be a simple filtrate of the blood plasma minus the serum proteins and fats similar to that passed through a gelatin filter from a colloid salt solution. The human tubular epithelium then enters into active selective reabsorption of water and substances to be conserved and probably adds to the concentrate urea, creatinine dyes, and organic iodide compounds.

Any consideration of the functions of the kidney must be concerned with the multiple extrarenal factors, blood plasma, electrolytes, nitrogenous waste,

colloids, intravascular and tissue pressures. Excretion of urine has been stopped when pressure in the ureter was raised to about 90 mm. Hg, with the blood pressure of 130 mm. Hg giving a differential pressure of about 40 mm. Hg. Dilution of the colloids in the plasma by adding urea, ammonium or potassium chloride or nitrate by mouth, or hypertonic glucose or injection of inert sugars and salt solutions by vein, draws water and salt from the tissues, lowers the pressure of the colloids, and inaugurates increased filtration and urine output. Changes in the circulation as congestive failure with anoxemia and in acute shock have been observed to produce oliguria or anuria. Water balance disturbances by dehydration by diarrhea, vomiting, or sweating decrease the urinary output considerably.

SIGNIFICANT CHEMICAL CONSTITUENTS OF BLOOD AND URINE

The blood plasma and dilute and concentrated urine content of the more common substances are of interest. For instance, the water percentages for blood plasma are 90 to 93 per cent as contrasted with 93 and 95 per cent for dilute and concentrated urine; the chlorides 0.370 Gm. per cent as contrasted with about 0.500 and 2,000 Gm. per cent; urea 0.030 Gm. per cent as contrasted with 2,000 and 6,000 Gm. per cent; uric acid 0.004 Gm. per cent as contrasted with 0.050 and 0.120 Gm. per cent; creatinine 0.001 Gm. per cent as contrasted with 0.075 and 0.750 Gm. per cent. Creatinine and creatine are related to the muscular development and are constant for the individual, hence only the percentages shift. Ammonia is increased with increased acidity of the urine, and a high protein diet increases urine acidity and ammonia and unidentifiable rest nitrogen. Ammonia increases also with nitrogen intake. Undetermined rest nitrogen amounts to between 2 and 12 per cent. A high protein intake of 100 Gm. per day yields a nitrogen output of 14 to 16 Gm., of which 80 to 90 per cent is urea. A low protein diet of 30 to 40 Gm. yields 4 Gm., of which 60 to 70 per cent is urea.

The inorganic salt metabolism and acid-base equilibrium concern chiefly sodium chloride as sodium and as chloride. Potassium, calcium, magnesium, carbonic acid, phosphates, and sulfates are of some significance, while copper, zinc, and foreign substances as thiosulfates and sulfocyanates play very minor roles. The sulfates though likewise unimportant are found in inorganic ethereal, organic acids, protein amino acid, cystine, cysteine, and neutral sulfur. According to Gamble, the bases in milliequivalents per liter as Na 142, K 5, Ca 5, and Mg 3, a total of 155, are balanced against the acid total of 155 made up of HCO_3 27, Cl 103, HPO_4 2, SO_4 1, organic acids 6, and protein 16.

The great reservoir of body water is the intracellular fluid; it represents about 50 per cent of the body weight. The intracellular fluid contains as its chief basic ion potassium and as its chief acid ions organic phosphates. The interstitial fluid makes up only 15 per cent of the body weight and differs in that sodium is its chief basic ion and chlorides constitute its chief acid ions. The interstitial fluid is more easily mobilized. The blood plasma amounts to only 5 per cent of the body weight but it is most active in water metabolism and presents evidence of very delicately controlled acid-base and electrolyte balances.

CIRCULATING BLOOD PLASMA

The kidney has two important functions to perform. It must regulate the salt and water exchanges so as to keep the osmotic pressure of the blood constant in addition to removal of the nonvolatile, nonprotein nitrogenous waste products of protein metabolism. The normal circulating blood plasma exhibits a delicate balance of electrolytes and colloids in equilibrium with those of the fluids of the interstitial tissues. A balance is normally established between the extruding intravascular blood pressure and the in-sucking osmotic pressure of blood colloids.

Blood plasma proteins are retained by the normal semipermeable glomerular membrane, while water and electrolytes filter through into Bowman's capsule in equimolar concentrations. The dilute glomerular filtrate is concentrated a hundred fold as the water, glucose, and some of the other constituents pass back through the epithelium of the convoluted tubules and the loop of Henle and through the walls of the distal capillary bed into the circulation. The increase in circulating blood plasma volume per se does not increase the urinary outflow.

Regulation of the kidney function by changes in the make-up of the blood has been demonstrated following electrolyte and colloid shifts. These slight changes in the blood plasma have been found promptly balanced by greater shifts of electrolytes and water from the interstitial tissues. Normal urinary constituents and other acid salts have been used to cause an increase of the plasma electrolytes to upset the electrolyte equilibrium and inaugurate an inflow from the tissues which in turn precipitates an increased urinary output.

CIRCULATORY FACTORS IN KIDNEY FUNCTION

Normal kidney units require an active adequate circulation as well as an expansive capillary bed in order to provide a sufficient filtration volume area. At the same time, however, there is a good deal of evidence that something more than physical processes is going on in the kidneys. Work is actually accomplished. Oxygen is utilized in renal metabolism, hippuric acid is synthesized from glycine and benzoic acid, and ammonia is formed. Utilization of oxygen by the isolated kidney in considerable amount with carbon dioxide formation has been demonstrated experimentally in the blood and by microanalyses. Oxygen consumption by the isolated kidney is considerably greater under conditions of increased activity. This has not been demonstrated in human beings. In fact, the ingestion of large amounts of fluid by normal individuals has been found by continental physiologists to produce a diuresis without a demonstrable increase in the general oxygen consumption. The forced fluid intake results in an increase in the minute output of the heart by an increase in the stroke volume with actually a decrease in the pulse rate and in the venous pressure and only a slight rise in the arterial blood pressure.

The renal parenchyma is in close physiologic relationship to the circulatory system and seems to be of the utmost importance in maintaining the constancy of blood plasma composition. The kidneys, by virtue of the

tremendous blood flow through them, constitute one of the four most vital capillary systems of the body. The vascular and renal systems are physiologically interdependent, the kidneys, perhaps playing the major role, but requiring nevertheless for this the maintenance of a quarter of the normal total blood flow. In the kidney, constriction of the afferent or the efferent arterioles of the glomerular tufts is possible and may affect the function oppositely.

In congestive failure of the circulation venous stasis increases the outflow from the increased volume blood plasma of water and salt into the tissue interstices. There are several other factors besides retention of salt and water in the tissues at work in causing oliguria. A decreased filtration through the glomerulus results from intraglomerular capillary pressure decrease with the usual fall in the systolic arterial pressure, in spite of the increased blood volume and the usually decreased blood colloid oncotic pressure. Tubular reabsorption remaining normal, the water content of the circulating blood increases. The general circulation may be improved by cardiac tonics and other measures, and diuresis results. The local circulation through the glomerular capillary tuft of the kidney may be influenced by drugs to dilate proximally or distally, or to contract likewise and in such changes of blood flow affect filtration or reabsorption.

Diuretic drugs, by changing the glomerular blood flow, upset the electrolyte balance³ of the blood plasma. This change seems to command a rapid mobilization of salts, and secondarily, fluids from the tissues, and transport to the kidney for excretion in a diuresis. The accumulated abnormal amounts of water and salts in the tissue interstices, the edema of the failing circulation, is thus dissipated. In the treatment of heart failure cases with oliguria the cardiac glucosides as digitalis improve the myocardial tone and the circulation thus evidently indirectly increasing the urinary output.

The primary regulation of kidney function by circulatory shifts *per se* has been repeatedly denied. Experimentally diuresis has been inaugurated and suspended with the maintenance of a constant blood pressure level. A pulse pressure change in the circulation may be the chief influence in modifying kidney function. Nonnenbruch felt that the filtration theory of renal function has exaggerated the significance of circulatory changes and the effect of arterial, capillary, and venous blood pressures on salt and water balance. He admitted that the abnormally high susceptibility of the renal arterioles to stimulation might result in contraction or dilatation, but this has not been proved experimentally as a significant factor in urine production. Many studies on the capillary bed in general physiology have revealed striking independence of the capillary flow of the size and of the tension in the arterioles. The status of the capillary bed, as a whole, may be significant. At the extreme limits the capillary flow must undoubtedly affect urinary output.

The effect of heat on the kidney, and particularly on its blood flow, has interested clinicians who have accomplished an increase in urinary output with diathermy in patients with anuria. Denervated kidneys have not responded in the same way. Cold did not produce exactly the opposite effects.

It seems that the effects are the results of reflexes. These reflexes in the case of heat, it is stated, do not result in increased circulation and yet there was increased urinary flow. In acute nephritis the high blood pressure is associated with oliguria and lower levels with increased urinary flow.

There may be quite a group of other variable factors effective in changing urinary flow, yet diuresis is not independent of an increased renal blood flow; in fact it needs it. The two go hand in hand, but we may have increased flow without diuresis, and diuresis without increased blood flow may be the result of the increased excretion of salts and water or decreased reabsorption. Chemical influences may cause a dilatation of the afferent or efferent glomerular arteriole and allow increased circulation or a constriction of the same with opposite effects. Frequently a prompt increase in the blood flow through the kidney is the primary response in its complicated activity.

INTERSTITIAL TISSUES

In order to explain the relatively ametabolic gross changes represented in water and salt shifts, the vastness of the capillary bed in the interstitial tissues all over the body, as well as in the kidney, must be taken into account. Krogh⁷ has estimated a capillary system of 10,000 meters in length and 63,000 square meters in surface area. In such a system the rapid exchange back and forth between the important electrolytes and fluids in the intercellular tissues and the blood plasma may easily be visualized as taking place rapidly as purely physical phenomena. Anoxemia or injury of the capillary wall of a significant segment of the system would cause edema formation. It has been estimated that the water content of the plasma changes about a hundred times a day, and that the whole water mass circulates throughout the body and makes a complete circuit probably six times each day.

The interstitial tissues and connective tissues form a very large extracellular reservoir or depot for the body fluids, salts, and other constituents. The capillary walls are single-cell semipermeable membranes through which water and electrolytes seem to pass back and forth freely. The colloid serum proteins, on the other hand, are held back completely in the normal glomerulus. It is evident, however, from Calvin's data on the blood plasma protein shifts during diuresis and following hydremic plethora, that in some areas the capillary walls permit the passage of serum proteins out of the tissue spaces into the blood plasma. The general rich capillary distribution makes possible the maintenance of equilibrium with relatively prompt return to the constancy of the blood plasma volume and its constituents.

NERVOUS AND HORMONE FACTORS^{10, 13}

Angioneurotic edema, allergic effusion, diabetes insipidus and its control with pitressin, myxedema, and desoxyepiandrosterone plethora are evidences that the nervous system and the glands of internal secretion play important, albeit largely unexplained, roles in water metabolism. Studies of the antidiuretic effect of pitressin in patients with diabetes insipidus indicate that the hormone pitressin acts directly on the kidney in some still rather

obscure way which is not related to the blood flow. At the same time there is following injection of pitressin much interstitial tissue effect which cannot as yet be elucidated.

Diabetes insipidus may result from injury to the posterior lobe of the pituitary or the hypothalamic region and tissues of the midbrain or the diencephalon. The posterior pituitary body is apparently linked physiologically with the great nuclear nerve origins as the vasomotor and other significant vegetative centers in the diencephalon. These centers are all susceptible to nervous humoral and hormonal stimulation. There is no proof that there is a decrease in pitressin in the blood stream to cause the thirst and diuresis of diabetes insipidus. The antidiuretic effect of pitressin persists in spite of the continued ingestion of large volumes of water and the addition of vasodilating drugs. The reported findings of abnormalities in the cells of the anterior lobe of the pituitary following diuresis and the apparent dehydrating effect of thyroxin have suggested the possibility of an anterior pituitary thyrotropic hormone disorder in myxedema.

The relatively recently discovered sodium, potassium, and plasma water balance disturbances in Addison's disease cases indicate that the adrenal cortical hormone is a significant factor in salt and water metabolism. The striking water storage effect of the synthetic hormone desoxycorticosterone is a fact that invites further investigations.

It is thus evident that the problems of water balance and water metabolism, edema formation, and diuresis have been extensively studied from almost every conceivable point of view.¹² Water intake, urinary output, blood plasma volume, and tissue fluid pressures have been shown to be most significantly concerned. In absolute water metabolism a delicate weighing is necessary to take into account the water that is lost in insensible perspiration, in exhaled air, and in the stools. Shifts in the individuals' body weight as determined by ordinary balances may record grossly the dissipation of the tissue edema, but the measured output of fluids through the kidney is the chief clinical index we have used. Normally the whole process from absorption of fluid through the gut, transport through the tissues and in the blood plasma to the kidneys, takes place without significant changes in the blood circulation time, volume, or pressures.

OUR STUDIES³⁰

Rehberg's¹⁴ creatinine clearance method was used at first in our laboratory in our studies of the mechanisms of diuresis by various substances in patients with congestive heart failure and edema. In spite of White and Monaghan's support, we were influenced by Shannon¹⁵ and Smith to determine also D-xylose and inulin clearances before and after the injection of mercurial and xanthine diuretics and digitalis glucosides. We have recorded the concomitant effects of drugs upon the blood plasma, electrolytes, and colloids, the urine volume and constituents, and the calculated renal glomerular filtration and tubular reabsorption. The evidence we obtained pointed to a primary effect directly upon the kidney units of the diuretic agents, such as are used in clinical medicine. The xanthines increased glomerular filtration, possibly

by afferent vasodilatation, and the mercurials decreased tubular reabsorption, possibly by efferent vasoconstriction or mild toxic decrease in permeability of the tubular epithelial cells. The possible pharmacodynamic renal effects were held responsible for slight but sudden and significant primary upsets in the very delicate electrolyte and colloid pressure balance in the blood plasma. Such slight shifts in turn, it was postulated, were promptly overcompensated for from the depots in the tissues in which there was edema. Salt, water, and proteins were drawn into the circulation. Electrolytes and fluid were then transported to the kidneys for excretion. A striking augmentation of the diuresis could routinely be produced by a combination of the two types of diuretics, and the clearance studies revealed combined effects with usually the mercurial effects, namely, decreased tubular reabsorption predominating. The tremendously augmented diuresis may not always be an unmixed blessing. This will be discussed more fully under the blood volume studies which contribute further argument.

SODIUM AND CHLORIDE STUDIES

The effect of mercurials, particularly salyrgan, upon sodium and chloride excretion and ratios has been recently restudied by several observers. Volhard's pupil, Weingarten,¹⁶ in support of the importance of the tissue salt and water status, so aptly designated "Vorniere" by his chief, held as indisputable the hydropigenous property of the chloride ion.

Bua,¹⁷ after the effects of salyrgan in diabetes insipidus, concluded that the presence of chlorine was the sine qua non requirement for the diuretic action of organic mercury salts. Simmert,¹⁸ observing the effect of diuresis produced by organic mercurial in patients with and without edema, found increases in the sodium chloride (determined also as chlorine) with concomitant rises in blood plasma sodium chloride (determined also as chlorine) along with decreases in hemoglobin and erythrocytes. Hitzenger and Englemann¹⁹ noted in salyrgan diuresis augmentation of excretion of many inorganic and organic substances, but particularly of sodium chloride.

The sodium ion, however, was incriminated in tissue fluid retention by Presser and Stahl.²⁰ These investigators found that the edema-producing effect of sodium could be set aside by a balanced combination of potassium, calcium and magnesium ions. This is the basis for the development of substitute for table salt for edematous patients. Bruman and Jenny²¹ established good correlation between sodium excretion and the urinary output. Siedek and Znekerkandl²² demonstrated greater retention of sodium than of chlorine in the edema fluid and transudates in the pleura and peritoneum in congestive heart failure patients. In diuresis of such edematous cardiac patients there appeared in the diuresate a conspicuous excess of sodium over chlorine.

In an attempt to elicit the initiating factors, and those at work during the first five to six hours of diuresis, Decherd³ determined the urinary Na/Cl ratios before, hourly during, and as a follow-up, each twenty-four hours for several days after each of 25 diuretic experiments with aminophyllin, mereupurin, and salyrgan. The blood sodium remained fixed, but the urinary sodium conspicuously increased, usually not in the tremendous proportions

of the chlorine excretion, so that the Na/Cl ratios as a rule decreased. Occasionally, however, the reverse was noted and the Na/Cl ratio increased. In these studies neither the mercurials nor the xanthines showed consistent or characteristic effects on the Na/Cl ratio.

If the volume of urine, with its contained sodium and chloride, passed during the control hours, was subtracted from the corresponding figures after injection of the diuretic, a fair approximation of the excess of these substances mobilized from the tissues during diuresis was obtained. By assuming the edema fluid to be an ultrafiltrate of blood plasma, the excess amount of sodium and chloride that should be excreted, with the excess urine excretion of the same composition as the tissue fluid, was calculated. The values for sodium determined on this basis showed a good agreement with those actually found, while the actual excretion of chloride was in most instances greatly in excess of the calculated values.

BLOOD SERUM PROTEIN STUDIES

Shally,²³ after some confirmatory physiochemical studies, accepted the conclusion of Nonnenbruch and others that changes in the colloid osmotic pressure did not explain the movements of water in diuresis. However, he found an absolute and relative increase in the albumin fraction, lowered and unchanged globulin, and a drop in fibrinogen at the onset of diuresis in all cases. He tried the effect of salyrgan on blood serum *in vitro* and concluded that in the concentration it appeared in the blood after injection salyrgan produced no change in the serum protein. He noted that at higher concentration there was flocculation. However, in this country, Bieter and Wright²⁴ have demonstrated, with ultramicroscope, changes in the blood serum of patients who had received therapeutic doses of salyrgan.

In recent studies in our department of biochemistry and in our laboratory for clinical research, Calvin and Deeherd^{25, 27-29} have demonstrated increases in the total circulating plasma protein, specifically albumin. They noted two different types of blood plasma volume changes following the exhibition of digoxin, aminophyllin, and salyrgan intravenously in patients with congestive heart failure and edema. The rapidity of the shifts of plasma proteins into and later out of the circulation suggested that there must be some degree of back flow of proteins as well as salts and water through the capillary walls in some parts of the capillary bed, possibly in the liver.

PLASMA VOLUME STUDIES

Meyer²⁶ postulated as the basis for diuresis a physico-therapeutic water-freeing effect upon the tissue colloids. The resulting hydremic plethora was held to dilate the renal vascular bed and account for the actual outpouring of urine. A study of the blood plasma volume shifts, using the new accurate method of Gregerson, Gibson, and Stead, and modified by Gibson and Evelyn with Evans blue dye 1824, seemed highly desirable. It was fortunate for us that D. B. Calvin, our biochemical experimentalist, had been working with this method of blood volume determination for four or five years. During the past year Calvin, together with Deeherd, undertook laboratory restudies of

the problems of diuresis in edematous congestive heart failure patients. In addition to establishment of the plasma volume shifts, the packed erythrocyte volumes, hemoglobin contents, albumin and total protein levels, as influenced by various diuretic agents, were determined.

The trends of plasma volume shifts seemed very definite. In a large series of experiments with the mercurial salyrgan (2 c.c. of 10 per cent solution) there was practically always a concentration in plasma volume that seemed to parallel diuresis. In two instances in which there was no diuresis and somewhat delayed diuresis there seemed to be a tendency for the plasma volume to increase slightly, but as soon as there was an increase in urinary flow there was a concomitant fall in the plasma volume.

Comparable studies using aminophyllin (7½ gr. or 0.5 Gm.) intravenously revealed a strikingly different plasma volume response. There was uniformly a conspicuous early rise in plasma volume. During the time of the most profuse diuresis there was a characteristic tendency for the plasma volume to return toward the initial level. As the aminophyllin diuresis continued, there was a shift in plasma volume apparently dependent, in part at least, upon the balance established between tissue fluid mobilization and urinary output. The end result was usually a plasma volume lower than that initially observed.

Digoxin, 2 mg. intravenously, in patients in congestive heart failure caused a shift in plasma volume apparently according to the promptness and degree of myocardial effect. When there was an immediate response, the plasma volume decreased sharply, as the satisfactory urinary output developed. When it was delayed, the plasma volume remained practically unchanged or increased slightly, until diuresis was inaugurated at which time the plasma volume decreased. Concomitant studies on the total circulating proteins indicate that there is a source of serum proteins, chiefly albumin, that is readily available and that the serum proteins may be promptly stored in the tissues.

COMMENTS

These most recent studies still leave many old questions as to the fundamental mechanisms of diuresis unanswered and open up some new ones. There is, however, some further evidence of a significant difference of the actions of mercurial diuretics as contrasted with xanthine diuretics. The relatively huge shifts in blood plasma volume following intravenous aminophyllin might account for some of the beneficent effects of such therapy in vascular disease. At the same time one might question the advisability of inducing such striking volume increases in patients with already abnormally high blood volumes and other evidences of congestive heart failure. In such cases repeated small injections and aminophyllin would seem to be more rational therapy. This suggestion may well be superfluous and the result of oversolicitousness, since no accidents indicative of such a mechanism have been recorded thus far. Then too, the combination drug, mercupurin, is much more often used in edematous cardiac patients, and under such circumstances the mercurial effect predominates, and as with salyrgan in the presence of good, or even fair, renal function there is from the beginning a shrinkage in the blood plasma volume.

The newer and more accurate methods offer new avenues of approach to the relatively old fundamental problems of the *modus operandi* of diuretics. The problems must, however, be vigorously prosecuted by every promising method to the end that the questions that have been raised by the survey of the subject may be answered. The answers to the questions will help in the further rationalization of diagnostic and therapeutic procedures.

REFERENCES

1. Addis, T., and Watanabe, C. K.: The Rate of Urine Excretion, *J. Biol. Chem.* 24: 203, 1916.
2. Nonnenbruch, W.: Diurese, *Klin. Fortbild.* 1: 697, 1933; *Wasserhaushalt, Der. Balneologie* 4: 17, 1937.
3. Gamble, J. L.: *Chemical Anatomy, Pathology of Extracellular Fluid*, Boston, 1939, Harvard Medical School Press.
4. Smith, Homer, W.: *The Physiology of the Kidney*, New York, 1937, Oxford Medical Publications.
5. Marshall, E. K.: The Secretion of the Urine, *Physiol. Rev.* 14: 133, 1934.
6. Richards, A. N.: Methods and Results of Direct Investigation of the Function of the Kidney, Baltimore, 1929, Williams & Wilkins Co.; *Proc. Roy. Soc. London, s. B.* 126: 398, 1938.
7. Krogh, A.: *Anatomy and Physiology of the Capillaries*, New Haven, 1922, Yale University Press.
8. Lewis, T.: *Blood Vessels of the Human Skin and Their Responses*, London, 1927, Shaw & Sons.
9. Cushny, A. R.: *Secretion of Urine*, ed. 11, London, 1925, Longmans, Green & Co.
10. Barcroft, J., and Straub, H.: The Secretion of Urine, *J. Physiol.* 41: 145, 1910.
11. Starling, W. H., and Verney, E. B.: The Secretion of Urine as Studied in the Isolated Kidney, *Proc. Roy. Soc. London, s. B.* 97: 328, 1924.
12. Eppinger, H.: *Zur Pathologie und Therapie des Menschlichen Odems*, Berlin, 1917, Urban & Schwarzenberg.
13. McCann, Wm. S.: Diuretic Action of Parathyroid Extract—(Collip) In Certain Edematous Patients, *J. A. M. A.* 90: 249, 1928.
14. Rehberg, P. B.: Studies on Kidney Function. I. The Rate of Filtration and Reabsorption in the Human Kidney, *Biochem. J.* 20: 447, 1926.
15. Shannon, J. A.: The Renal Excretion of Creatinine in Man, *J. Clin. Investigation* 14: 403, 1935.
16. Weingarten, R.: Über Kochsalzersatzmittel, *München. med. Wehnschr.* 79: 965, 1932.
17. Bua, F.: Über die Wirkungsart der Quecksilberdiurese bzw.-Antidiurese, *Klin. Wehnschr.* 14: 934, 1935.
18. Simmert, H. U.: Über die diuretische Wirkung organischer Quecksilberverbindungen, *Klin. Wehnschr.* 14: 530, 1935.
19. Hitzenger, K., and Englemann, F. L.: Über das spezifische Gewicht des Harnes bei Saltyrgandiurese, *Ztschr. f. klin. Med.* 129: 290, 1935.
20. Presser, H., and Stahl, R.: Untersuchungen ueber hydropigene Ionenwirkungen auf lebende Gewebe mittels Durchströmung im Tierversuch, *München. med. Wehnschr.* 80: 559, 1933.
21. Bruman, F., and Jenny, F.: Der Einfluss der Ernährung auf den Stoffunisatz bei der Arbeit, *Deutsches Arch. f. klin. Med.* 177: 527, 1934.
22. Siedek, H., and Zuckerkandl, F.: Die Bedeutung des Natrium—Chlor—Quotienten im Harn beim "Volhardschen" Wasserversuch, *Klin. Wehnschr.* 14: 1428, 1935.
23. Shally, H. O.: Veränderungen der Bluteiweisskörper bei der Saltyrgandiurese, *Deutsches Arch. f. klin. Med.* 177: 368, 1934.
24. Bieter, R. N., and Wright, H. N. G.: Fundamental Studies on the Pharmacology of Mercury Diuretics. In the Kidney in Health and in Disease, Berglund, et al., Philadelphia, 1935, Lea & Febiger, p. 701.
25. Calvin, D. B.: *Am. J. Physiol.* In Press.
26. Meyer, H. H., and Gottlieb, R.: Die experimentelle Pharmakologie, Berlin, 1933, Urban & Schwarzenberg, p. 489.
27. Decherd, G. M., Jr., Calvin, D. B., and Herrmann, G.: Blood Plasma Volume Changes Following the Administration of Diuretics, *J. Clin. Investigation* 19: 778, 1940.
28. Calvin, D. B., Decherd, G. M., Jr., and Herrmann, G.: Response of Plasma Volume to Diuretics, *Proc. Soc. Exper. Biol. & Med.* 44: 529, 1940.
29. Calvin, D. B., Decherd, G. M., Jr., and Herrmann, G.: Plasma Protein Shifts During Diuresis, *Proc. Soc. Exper. Biol. & Med.* 44: 578, 1940.
30. Herrmann, G. R., and Decherd, G. M., Jr.: Further Studies on the Mechanisms of Diuresis With Especial Reference to the Action of Some Newer Diuretics, *J. Lab. & Clin. Med.* 22: 767, 1937.

THE FUNCTIONS OF THE CAROTID AND AORTIC BODIES*

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THE functions of the carotid and aortic bodies have been covered by four reviews within the past year,^{11, 13, 17c, 29} and no striking discoveries have been made since the last of these was published. My justification for acceding to the request for another review at this time is the hope of adding something to the understanding of this special branch of physiology by treating it from a different viewpoint, viz., that of the historical perspective of the subject, the persons involved, their reasons (as far as I know them) for doing what they did, and the influence of the various contributions on the development of the subject. This is not the least interesting and important part of this recent chapter in experimental medicine, and although some of the persons and factors involved have already been mentioned,^{28c} none of the existing reviews have been compiled so that a reader not familiar with the subject can trace its development.

Prior to 1930 the functions of the carotid and aortic bodies were unknown. This was not a very serious lack because the only interest in these structures lay in the rare occurrence of a tumor of the carotid body and the still rarer performance of an investigation of the morphology and embryology of that tissue. Such studies² left unsettled the question of whether the carotid body is a nervous structure (a ganglion or a paraganglion), a secreting gland of the chromaffin type, a peculiar cavernous vascular structure (a glomus), or something else (simply a body). Numerous attempts had been made to demonstrate a physiologically important secretion in the carotid body or in tumors arising from it, but none of these were very successful.

The event that served to direct attention into productive channels was Hering's discovery¹⁶ that the slowing of the pulse which had long been known to occur when deep pressure is exerted in the neck is not due to mechanical stimulation of the cardioinhibitory fibers of the vagus, as had been supposed for many years, but to stimulation of special receptors localized in the expanded region at the origin of the internal carotid artery. He called this the *carotid sinus*, and the term has come into general use; it is perhaps noteworthy that Hering's original intention is said to have been to call it the *carotid bulb*, but he decided against this because of the previous existence of another bulbo (oeno) cardiac reflex. He found that the afferent pathway for this new reflex is the first (pharyngeal) branch of the glossopharyngeal nerve (now called the sinus, intercarotid, or Hering's nerve) and that the reflex consists not only of cardiac inhibition but also of decreased vasomotor activity, so that it is completely analogous to the depressor reflex from the aorta described sixty years earlier by Ludwig and Cyon. The two carotid sinus nerves and the two depressor nerves were found by Hering to constitute a functional unit, any two, or, given sufficient time, even one, of the four being capable of carrying on an adequate

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degree of functional activity; but when all are cut, heart and vasomotor center become excessively active and persistent tachycardia and hypertension result. Because the influence of these reflexes is to restrain the heart and the vasomotor center, Hering called the four nerves which carry them the "Blutdruckzügler," or check reins of blood pressure. Subsequent workers have called them the "Nerfs Frenateurs" (Heymans) and the "buffer nerves" (Samson Wright), implying the same sort of moderator activity. It is noteworthy, however, that since these nerves are now known to carry other impulses which are stimulant, these descriptive terms, while widely used, are not strictly accurate.

The work of Hering introduced a new element into discussions of the regulation of the circulation. It attracted widespread attention because it presented a new viewpoint and opened a vista of possibilities previously unknown. Thus, the conception that the vasomotor center possesses a high intrinsic "tone" and is kept from producing maximal vasoconstriction only by afferent inhibitory nerve impulses aroused in special stretch receptors in the carotid sinuses and aortic arch has obvious implications to sustained hypertension or hypotension, syncope, and other clinical problems.³⁴ The conception that the cardioinhibitory center is activated, not by changes in the pressure or volume of flow of blood through it, but solely by these same afferent nerve impulses, has important implications with regard to the mode of regulation of the activity of nerve cells, which is one of the most important questions in all physiology. The technical difficulties involved in experiments such as those of Hering proved, however, to be considerably greater than was anticipated, and for several years no significant progress was made.

During this time J. F. Heymans, Professor of Pharmacology in the University of Ghent, and his son Corneille were engaged in an investigation of various physiologic problems by means of the crossed-circulation technique. In one remarkable set of experiments²⁰ they found that the respiratory movements of the head of a dog—entirely separated from the trunk save for the vagodepressor nerves and kept alive by anastomosis into the carotid-jugular circulation of a donor animal—could be depressed by a rise and stimulated by a fall in blood pressure in the trunk of the animal, and could be stimulated by asphyxia, anoxemia, or hypercarbia, depressed by overventilation or increased oxygenation of the blood in the trunk. The reflex nature of these phenomena was proved by their absence when the vagodepressor nerves were cut. By further refinements and amplifications of their technique, they showed that the reflexes responsible arise from the aortic arch. They concluded that such reflexes are a factor of importance in the control of respiration.

The report of these studies was published in 1927. Meanwhile Danielopolu and his co-workers in Bucharest,⁵ stimulated by Hering's discovery, had carried out a number of experiments on dogs and human beings and had found that reflexes from the carotid sinuses can affect respiration as well as circulation. They claimed that reflexes can also be aroused here by chemical agents (increased carbon dioxide tension) as well as by changes in pressure. Moissejeff²⁴ also described respiratory effects from the carotid sinus. The experimental evidence presented by these workers was not very impressive, and while they undoubtedly antedated Heymans in calling attention to the existence of respiratory reflexes

from the carotid sinus region, their work might have had little influence upon subsequent events had it not come at a time when J. F. Heymans and C. Heymans had just finished the studies from which they concluded that reflexes from the aorta, aroused by changes in pressure or chemical composition of the blood, constitute an important and hitherto unrecognized factor in the control of respiration. The statements claiming a similar, though less well-defined, function for carotid reflexes therefore constituted a challenge to the Belgian workers which the latter were not slow to accept. It is noteworthy that Heymans, whose name is now inseparably linked with the carotid sinus as one of its most effective advocates and who was awarded the 1938 Nobel Prize in Physiology and Medicine in recognition of his contributions to better understanding of the part played by these reflexes in the control of respiration and the influence of drugs upon it, began his work in that field in hopes of disproving the things he subsequently proved. Incidentally, a similar motive has activated a large proportion of the others (including myself) who have followed Heymans in investigating carotid reflexes to respiration.

During the same period de Castro,⁷ in Cajal's laboratory in Madrid, was carrying out a series of morphologic studies of the nerve structures involved in this new reflex of Hering. He found that nerve endings of a peculiar type are present in the adventitial coat of the internal carotid artery in the expanded region near its origin, viz., in Hering's carotid sinus. He also found an abundance of similar nerve structures in the carotid body, and in his first paper^{7a} he concluded that this structure is probably a secreting gland innervated by the glossopharyngeal; his studies led him to believe that the sinus nerve of Hering is a branch of the vagus, not of the glossopharyngeal, the latter being concerned only with the carotid body (or gland). Further study,^{7b} however, led him to modify his conclusions; the nerve system of the carotid body now seemed to be afferent at least in part, and he suggested that it is probably concerned in the regulation of blood pressure, its function being similar to, though less striking than, that of the carotid sinus^{7b, p. 364}; he also suggested^{7b, p. 370} that it might be a "sensory organ specialized to perceive certain qualitative changes in the blood." Several other workers^{8, 21} concluded that the carotid body is the site of origin of Hering's reflexes, not the carotid sinus, but it was not until the work of Heymans and his collaborators had shown clearly for the first time that there are two distinct sets of carotid reflexes, viz., those aroused by pressure and those aroused by chemical factors, that the second suggestion of de Castro led to productive experimental studies and was proved correct. Heymans and his co-workers were able to separate the two types of sensitivity, retaining that to chemical influences while removing that to pressure, or the reverse, and other workers succeeded in demonstrating that the chemically sensitive region in the dog is located in the carotid body. (The literature on this aspect of the subject is cited elsewhere.²⁰) Thus, the true function of this structure was finally proved to be none of those previously attributed to it, but instead, a completely unique one, i.e., translation of certain chemical changes in the blood into afferent impulses stimulant to the respiratory, vasomotor, and cardioinhibitory centers. These results stimulated other studies of similar tissue elsewhere, and a number of aggregates of cells resembling the carotid body morphologically were found

in the aortic region.^{2, 26} One of these was shown by the physiologic studies of Comroe³ to be the probable site of the aortic chemoreceptors; it receives blood from a small branch of the transverse aorta in the dog, of a coronary artery in the cat, and nerve fibers from it join the vagodepressor trunk at about the level of emergence of the recurrent laryngeal. The function of the other similar structures in the thorax and in the coccygeal region still remains unknown.

The papers by Heymans and his co-workers dealing with carotid reflexes to the respiratory center did for respiratory physiology what Hering's papers had done for circulatory physiology, i.e., they introduced a new element into discussions of the control of respiration and led to a critical re-examination of beliefs that had gone unchallenged for many years. At that time there was no doubt among physiologists that the major element in the automatic control of respiration was the effect of metabolic products directly upon the cells of the center and that afferent nerve impulses were nothing more than a nonessential factor. There had been some disagreement as to the identity of the chemical stimulus, but this was compromised to the satisfaction of most physiologists when Haldane (who was the first to demonstrate the very great effectiveness of carbon dioxide) accepted the view that the common denominator in all aspects of the chemical control of breathing is hydrogen-ion concentration in the blood.¹⁵ On this basis there were some difficulties in explaining the respiratory effects of changes in blood pressure (the respiratory depression or apnea produced by a sudden rise and the hyperpnea produced by a sharp fall), but these found a satisfactory solution in Rosenthal's conception,²⁷ as revived by Winterstein²⁸ and Gesell,¹² to the effect that it is the concentration of chemical stimuli within the cells of the center that regulates their activity, and since these cells are alive and have a metabolism of their own, their activity can be modified by changes in the rate at which products of their own metabolism are removed by the blood stream. The apnea produced by an intravenous injection of adrenalin or the hyperpnea produced by sudden hemorrhage, histamine injection, or any other sharp fall in blood pressure was an important piece of evidence in favor of this conception of respiratory control. The hyperpnea of anoxemia, which at first glance seems difficult to explain in terms of carbon dioxide tension or hydrogen-ion concentration in the arterial blood (for both of these are apt to be reduced by anoxemia), was attributed either to a sensitization of the center to its normal stimulus (carbon dioxide or pH) by anoxemia,¹⁵ or to accumulation of lactic acid within the cells of the center because of incomplete oxidation of metabolic products therein.¹²

Heymans and his colleagues presented evidence which, if fully substantiated, would have discredited entirely the Rosenthal-Winterstein-Gesell conception of respiratory control and would have necessitated radical revision in Haldane's. They showed¹⁷ that a rise in intracarotid pressure causes reflex inhibition of respiration, and this, coupled with the similar reflexes²⁰ aroused by a rise in aortic pressure, led them to suspect that the respiratory effects of changes in blood pressure might be due entirely to reflexes from the carotid sinuses and aortic arch—a suspicion which they verified by seemingly irrefutable experimental evidence and concluded that the activity of the cells of the respiratory center is not demonstrably influenced by changes in its blood supply, at least over

the physiologic range. They also¹⁸ found that an anoxic hyperpnea (and hypertension), whether produced by decrease in the oxygen tension in the blood or by the addition of cyanide to it, is mainly, if not exclusively, reflex in origin; section of the carotid sinus and aortic (depressor) nerves greatly reduced or completely abolished the stimulant effects of anoxemia on respiration and circulation, thus indicating that accumulation of products of incomplete oxidation in the center cannot be the true explanation of these effects. Even changes in carbon dioxide tension and pH in the arterial blood were found to produce their effects in part through these reflexes. Although it was never claimed that the respiratory effects of these changes are dominantly or exclusively due to reflexes from chemoreceptors (that claim was reserved for anoxemia and for drugs such as cyanide, nicotine, and lobeline), it was nevertheless reported that reflexes play a part in them. Thus the extreme sensitivity of the respiratory center to minute changes in the carbon dioxide tension or pH in the blood, which was perhaps the best-known and most firmly established fact in the entire field of respiratory physiology, came under suspicion, and with it the entire Haldane conception of the control of breathing. (As a matter of fact, Heymans did not commit himself at the time as to whether the chemoreceptors or the center are more sensitive to carbon dioxide and pH. It was not until 1939 that his position on this point was clearly stated, and then he held^{17c} that the chemoreceptors are more sensitive, act more quickly, and are responsible for a greater share of the total response to these stimuli than can be said of direct effects on the center.)

These papers naturally aroused a great deal of interest among all who were working in the field of respiratory physiology. Since we²⁹ have recently reviewed the literature on this subject from the viewpoint of the adequacy of the experimental evidence, it is unnecessary to recapitulate here the list of contributions that have followed. For the sake of historical perspective, however, it is worth noting that, with the appearance of Heymans' first papers, these reflexes came to be a major interest in the two laboratories in this country (Gesell's and our own) in which the workers had gone furthest in committing themselves to the Rosenthal-Winterstein-Gesell conception of respiratory control, which now seemed to be completely invalidated by Heymans' finding that the respiratory center is unaffected by changes in its blood supply. The resulting contributions from Gesell's laboratory, as summarized elsewhere,¹³ leave that group in substantial agreement with Heymans in all essential respects, i.e., that the responses of respiration to changes in blood pressure are due to reflexes from the pressoreceptors of the carotid sinuses and aortic arch, rather than to changes in the blood supply of the center, that the sensitivity of the chemoreceptors to carbon dioxide and pH is of the same order as that of the center, and that the chemoreceptors contribute an important share to the regulation of respiration under all circumstances. The work in our laboratory also led from the start^{28a} to agreement with Heymans about the existence of the two sets of reflexes (from pressoreceptors and chemoreceptors) and the dominant importance of the latter in the respiratory and circulatory stimulant effects of anoxemia. From the start, however,^{28a} we have been able to show that the respiratory center is not always as insensitive to changes in its blood supply as it was in Heymans' experiments, and among the factors that might be concerned

in such variations an outstanding one seemed to be the state of the finer blood vessels supplying the center. To investigate this relationship, an examination of the intrinsic control of the cerebral circulation was begun and is still in progress; the bearing of the results so far obtained on the above question has been considered elsewhere.³² From these experiments we have come to believe that the insensitivity of the respiratory center to changes in its blood supply in Heymans' experiments was due to fortuitous experimental circumstances,³³ and, therefore, does not warrant the general conclusion that the Rosenthal-Winterstein-Gesell conception of respiratory control is invalid. We also differed with Heymans from the start in believing that the respiratory center is so much more sensitive than the chemoreceptors to carbon dioxide that the normal control of breathing is probably accomplished by the center alone, without involving the chemoreceptors. Subsequent experimental evidence has only served to strengthen this belief.²⁹

The present status of the questions raised by Heymans' work, in briefest summary, is as follows: There is general agreement about the existence of two sets of reflexes in carotids and aorta, the one activated by changes in arterial pressure, the other by changes in chemical composition of the arterial blood; the receptors responding to pressure (pressoreceptors) are located in the adventitial coat of the carotid sinus and aortic arch, those responding to chemical changes (chemoreceptors), in the carotid and aortic bodies; the impulses from both sets of receptors are carried by the same nerves (the sinus and depressor) and affect the same structures (the respiratory, vasomotor, and cardio-regulatory centers in the medulla), although for the most part in opposite directions, i.e., impulses from the pressoreceptors lead to *decreased* activity of the respiratory, vasomotor, and cardioaccelerator centers (and increased activity of the cardioinhibitory), while impulses from the chemoreceptors cause *increased* activity of all these centers (including the cardioinhibitory in the case of strong stimulation of the carotid chemoreceptors of the dog). There is also general agreement that the chemoreceptors can be stimulated by each of the chemical factors that have long been associated with the control of respiration and circulation, namely, increased carbon dioxide tension or hydrogen-ion concentration, or decreased oxygen tension, as well as by a variety of drugs and poisons (cyanides, sulfides, nicotine, lobeline, coniine, sparteine, choline and its nicotinic derivatives, acetaldehyde, potassium salts, dinitrophenol, dinitroresol, and others). It is also agreed that the reflexes from the pressoreceptors (which are those originally described by Hering) are of utmost importance to the control of the circulation, but of comparatively little moment to the regulation of respiration, whereas the chemoreceptor reflexes are of major importance to respiration, but are relatively unimportant to the circulation, with one significant exception, viz., the hypertension of anoxemia.

When it comes to an evaluation of the part played by these reflexes in the adjustment of the organism to its environment, differences of opinion are encountered. As indicated elsewhere,²⁹ these seem to us to be due to inadequacy of experimental evidence relative to the conclusions drawn, for the data needed for this purpose must be quantitative, and the step from the qualitative information that suffices to demonstrate the existence of these reflexes to the

quantitative data required to evaluate their importance is long and difficult. It is clear, however, that the hyperpnea and hypertension of anoxia are (as Heymans claimed from the start) due more to reflexes from the chemoreceptors than to direct effects on the center, and in my opinion, the burden of evidence indicates that anoxia is purely depressant to nerve cells, any stimulant effects being due to reflexes; this view is not shared by Gesell¹² or by Dautrebande,⁶ but it is supported by recent work done in Heymans' laboratory.¹ As for the part played by chemoreceptor reflexes in the body's responses to exercise or increased carbon dioxide tension or hydrogen-ion concentration, existing information²⁹ leads me to conclude that this is negligible under normal circumstances, but it can become very important when the sensitivity of the center to carbon dioxide is abnormally low, as in narcotic poisoning; this belief is not concurred in by Heymans and Bouckaert,^{17c} Gesell,¹² or von Euler and his collaborators.¹⁰ The point of fundamental importance here is the relative sensitivity of the center and of the chemoreceptors to carbon dioxide or pH, and the only quantitative data now available are those^{4, 20, 21} which have led us to the above conclusion. Another point of importance in deciding on the physiologic significance of these reflexes is the nature of the stimulus by which they are activated; it is perhaps inevitable that attempts should be made to simplify the situation by finding a common denominator for all chemoreceptor stimulations, and different workers have suggested increased acidity,¹³ anoxia,⁴ acetylcholine,²³ and potassium⁹ in this connection. In view of the diverse nature of the substances that have been proved capable of stimulating the chemoreceptors,²⁹ such attempts do not seem very profitable at this time; it seems better to conclude simply that these structures are so organized that they give rise to nerve impulses when the chemical equilibria of the cells are sufficiently disturbed, and that such disturbances can be brought about in a number of different ways. The question of the identity of the stimulus to the chemoreceptors is likely to assume greater importance in the near future in view of the recent work in Krogh's laboratory^{22, 25} leading to the conclusion that the chemical control of respiration is accomplished by carbon dioxide acting as such, not by virtue of changes in acidity produced by it. If this conclusion is correct, the respiratory effects of changes in pH of the blood could be attributed either to the changes in carbon dioxide tension produced by them or to reflexes from the chemoreceptors; perhaps these are the only parts of the respiratory regulating system that respond directly to the hydrogen ion.

My opinion of the significance of these structures agrees with that of Marshall and Rosenfeld,²³ who suggested that they represent a survival of a primitive type of control found in water-breathing animals. As noted elsewhere,^{4, 25b, 29} this conception is supported both by existing information concerning the regulation of respiration in fish²² and by the derivation of the carotid (and probably aortic) bodies from the same tissues that give rise to the branchial (gill) arterial system in the embryo.² It accounts for the great resistance of these receptors to the depressant effects of anoxia, for this is characteristic of embryonic tissues in general, and the carotid and aortic bodies presumably are relatively primitive, undifferentiated structures; perhaps their ability to be stimulated by anoxia and by great increases in carbon dioxide

tension and acidity, as well as by drugs and poisons that depress many other tissues, depends simply on their resistance to depression by these agents. This conception appears to be in better accord with available information than the alternative suggestion¹³ that these structures have the same functional characteristics and significance as the neurones of the medullary centers.

SUMMARY

The carotid and aortic bodies are structures whose specialized function it is to respond to changes in the chemical composition of the arterial blood by setting up afferent impulses which enter the central nervous system with the glossopharyngeal and vagus nerves, respectively.

The nerve impulses in question are stimulant to the medullary centers (respiratory, vasomotor, and, in the case of the carotid body of the dog, cardio-inhibitory). The impulses from the stretch receptors of the carotid sinuses and aortic arch (carried by the same nerves) are inhibitory to these centers (except the cardioinhibitory, which is stimulated by them).

The chemically sensitive receptors (chemoreceptors) can be stimulated by anoxemia, asphyxia, increased carbon dioxide tension, or increased hydrogen-ion concentration, or by a variety of drugs and poisons which have no common chemical or physiologic attribute although the list includes inhibitors of oxidations (cyanide, sulfide) and substances with nicotinic properties (nicotine, lobeline, coniine, and choline and its derivatives).

Reflexes from these structures are responsible for much, if not all, of the stimulant effects of anoxia on respiration and circulation. They probably are not concerned in the normal control of respiration, the sensitivity of the chemoreceptors to carbon dioxide being much less than that of the centers unless the latter is depressed; in that event these reflexes become an important factor in maintaining respiration.

An explanation that is in accord with existing information on the subject is that the chemoreceptors represent a survival in relatively undifferentiated form of a reflex mechanism originally developed for a water-breathing ancestral form. The ability of these structures to set up a strong reflex stimulation to respiration when exposed to an environment that would depress or paralyze nerve cells (severe anoxia, very high carbon dioxide tension or acidity, deep narcosis) is probably related to their primitive status and responsible for much, if not all, of their value to the organism.

REFERENCES

1. Bouckaert, J. J., Grimson, K. S., and Heymans, C.: *J. Physiol.* 95: 11, 1939.
2. Boyd, J. D.: Publication No. 479, Carnegie Institute, Washington, D. C., 1937.
3. Comroe, J. H., Jr.: *Am. J. Physiol.* 127: 176, 1939.
4. Comroe, J. H., Jr., and Schmidt, C. F.: *Am. J. Physiol.* 121: 75, 1938.
5. Danielopolu, D., Marcu, L., and Proca, G. G.: *Klin. Wehnschr.* 10: 2176, 1931.
6. Dautrebande, L.: Volume jubilé en l'honneur du Prof. J. Demoor, 1937, p. 133.
7. De Castro, F.: *Trav. Lab. Rech. Biol., Univ. Madrid.* (a) *Rev. Trimest. Micrograf* 24: 365, 1926; (b) 25: 331, 1927-28.
8. Drüner, L.: *Deutsch. med. Wehnschr.* 51: 559, 1925.
9. von Euler, U. S.: *Skandinav. Arch. f. Physiol.* 80: 94, 1938.
10. von Euler, U. S., Liljestrang, G., and Zotterman, Y.: (a) *Skandinav. Arch. f. Physiol.* 74: 101, 1936; (b) *Ibid.* 83: 132, 1939.
11. Gellhorn, E., and Lambert, E. H.: *Illinois Med. & Dent. Monograph* 2: 1939.
12. Gesell, R.: *Physiol. Rev.* 5: 551, 1925.

13. Gesell, R.: *Ann. Rev. Physiol.* 1: 185, 1939.
14. Gollwitzer-Meier, K.: *Pflüger's Arch. f. d. ges. Physiol.* 234: 342, 1934.
15. Haldane, J. S.: *Respiration*, New Haven, 1922, Yale University Press.
16. Hering, H. E.: *Pflüger's Arch. f. d. ges. Physiol.* 206: 721, 1924; *Karotissinusreflexe auf Herz und Gefässe*, Dresden, 1927.
17. Heymans, C., and Bouckaert, J. J.: (a) *Rev. belge se. méd.* 1: 507, 1929; (b) *J. Physiol.* 49: 254, 1930; (c) *Ergebn. d. Physiol.* 41: 28, 1939.
18. Heymans, C., Bouckaert, J. J., and Dautrebande, L.: *Arch. internat. de pharmacodyn. et de théor.* 39: 400, 1930; 40: 54, 1931.
19. Heymans, C., Bouckaert, J. J., and Regniers, P.: *Le Sinus Carotidien*, Paris, 1933.
20. Heymans, J. F., and Heymans, C.: *Arch. internat. de pharmacodyn. et de théor.* 33: 273, 1927.
21. Jacobivici, J., Nitescu, I. I., and Pop, A.: *Compt. rend. Soc. de biol.* 98: 732, 1928.
22. Krogh, A.: *Comparative Physiology of Respiratory Mechanisms*, Philadelphia, 1940, University of Pennsylvania Press.
23. Marshall, E. K., Jr., and Rosenfeld, M.: *J. Pharmacol. & Exper. Therap.* 57: 437, 1936.
24. Moissejeff, E.: *Ztschr. f. d. ges. exper. Med.* 53: 696, 1926-27.
25. Nielsen, M.: *Skandinav. Arch. f. Physiol.* 74: suppl. 10, p. 88, 1936.
26. Nonidez, J. F.: *Am. J. Anat.* 57: 259, 1935; *J. Anat.* 70: 215, 1936.
27. Rosenthal, J.: *Hermanns Handbuch d. Physiol.*, vol. 4, part 2, p. 270. Leipzig, 1880.
28. Schmidt, C. F.: (a) *Am. J. Physiol.* 102: 94, 1932; (b) *Macleod's Physiology in Modern Medicine*, ed. 8, St. Louis, 1938, The C. V. Mosby Co., pp. 498-516; (c) *Scientific Monthly* 49: 576, 1939.
29. Schmidt, C. F., and Comroe, J. H., Jr.: *Physiol. Rev.* 20: 115, 1940.
30. Schmidt, C. F., Comroe, J. H., Jr., and Dripps, R. D., Jr.: *Proc. Soc. Exper. Biol. & Med.* 42: 31, 1939.
31. Schmidt, C. F., Dumke, P. R., and Dripps, R. D., Jr.: *Am. J. Physiol.* 128: 1, 1939-40.
32. Schmidt, C. F., and Hendrix, J. P.: *Proc. A. Res. Nerv. & Ment. Dis.* 18: 229, 1938.
33. Schweitzer, A., and Wright, S.: *Quart. J. Exper. Physiol.* 28: 33, 1938.
34. Weiss, S., and Baker, J. P.: *Medicina* 12: 297, 1933.
35. Winterstein, H.: *Pflüger's Arch. f. d. ges. Physiol.* 187: 293, 1921.

EPILEPSY: NEWER METHODS OF INVESTIGATION AND TREATMENT*

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THE earliest medical writers speculated about the cause of seizures in epilepsy and prescribed meticulous treatments. From the time the well-known treatise on the subject was written by Hippocrates or one of his followers until the present time is a span of some 2,400 years. During nine-tenths of this period doctors were interested not in newer treatments but in older ones. They wanted to know what Hippocrates, Galen, and Aretaeus had to say about the Falling Sickness. Advances in knowledge have been prominent in three periods: First, about the end of the seventeenth century, Thomas Willis in England and Boerhaave in Holland began to look with their own eyes at the problem of epilepsy. Second, the latter half of the nineteenth century stands out because of the animal experiments of Kussmaul and Tenner, and Frisch and Hitzig, the school of English clinicians led by Hughlings Jackson, Gowers, Reynolds, and Sieveking, and the first use by Locock of an effective anticonvulsant drug, bromide. Third, is the present "new methods" period.

The progress of research in epilepsy has, in general, followed the development of clinical and laboratory techniques. Because physicians realized that seizures arose in the brain, anatomy, at first gross and then microscopic, was expected to answer the riddle of epilepsy. However, as long ago as 1859 Kussmaul and Tenner said: "The judicious physician will relinquish the hope that pathological anatomy is destined to give an explanation of the nature and seat of epilepsy, and he will only expect that result from the progress of the experimental physiology of nerves." Events have confirmed this prediction. It is now realized that demonstrable structural abnormalities of the brain are the results of seizures or else only a contributing cause. There may be certain patients, for example, adults with a tumor of the frontal or parietal lobe, in whom the pathology is the sole cause of seizures, but such cases constitute only an insignificant fraction of the total number of patients who are subject to seizures.

If the primary disorder is not anatomic, but a disturbed function of nervous tissues, then the specialized and intricate techniques used in chemistry, physics, and physiology must be called upon. However, medical science does not always advance along the lines of logical development; it may start from an unorthodox chance observation. Twenty years ago attention was attracted to an osteopathic practitioner who was treating patients with seizures by means of fasting, with, in some instances, dramatic results. The observation was empirical but sound, and the beginning of the third period mentioned may properly be marked—

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Conklin, 1920. Geyelin¹ believed that the beneficial results of fasting may be due to the acidosis induced, and carried through a great volume of laboratory examinations which, unfortunately, he never published. Wilder² suggested that the acidosis of fasting could be induced also by a ketogenic diet which could be maintained for a longer period than fasting. Other workers at the Mayo Clinic,³⁻⁶ in Boston,⁷ in Baltimore,⁸ and elsewhere have demonstrated that ketosis is accompanied by definite improvement in many (about one-half) of the children treated. An inorganic acidosis, induced by ingestion of hydrochloric acid,⁹ of acid-forming salts, or by the inhalation of air containing 10 per cent carbon dioxide¹⁰ also proved to be temporarily effective in stopping petit mal. Rosett¹¹ and Foerster¹² precipitated attacks by the opposite condition of hyperpnea.

Changes in the acid-base balance of the organism are attended by widespread alterations in other physicochemical and physiologic relationships. The simple act of overventilation, which so readily precipitates petit mal, causes a constriction of cerebral arterioles, with resulting decrease in cerebral blood flow,¹³ and also alters the electrical conductivity of nervous tissues¹⁴ and the oxygen dissociation curves of the blood. Although the oxygen saturation of the arterial and internal jugular blood of epileptic patients is not abnormal, a sharp reduction in the oxygen tension of inspired air will bring on petit mal attacks,¹⁵ and a temporary increase in the oxygen tension up to twenty times normal will hinder attacks.¹⁵ However, prolonged exposure to still higher tensions will precipitate convulsions.

Working on the hypothesis that epileptics have an incomplete drainage of spinal fluid with resulting pressure on the cortex from the accumulated fluid, Fay¹⁶ proposed treatment by means of dehydration. Though the hypothesis was faulty, as others^{17, 18} have shown, the demonstration of positive effect of dehydration on seizures was valid. That the effect was chemical and not, as Fay had argued, hydrostatic, was indicated by the demonstration by McQuarrie¹⁹ that seizures could be precipitated by retention of body fluids induced by the injection of pitressin in association with a high water intake; if normal saline were substituted for the water, seizures did not result.

That convulsions in animals and patients follow the injection of an overdose of insulin has been known since that hormone was isolated, but the neurochemistry involved was conjectural. There seemed to be no pressing reason to pursue the problem until it was discovered that the induction of severe hypoglycemia seemed to improve the mental condition of patients with schizophrenia. Then therapeutic convulsions, induced either by insulin or metrazol or electrical shock, became fashionable, and gradually physiologists and physicians are beginning to grasp the heaven-sent opportunity to study the chemistry involved in convulsions induced in human beings. Apparently with the exhaustion of the glucose of the brain, glucose and oxygen consumption is reduced,^{20, 21} and in the eviscerated animal the only thing which will restore cerebral activity is glucose itself, and not one of its derivatives.²²

Although epileptics who have diabetes seem to have fewer seizures when the diabetes is worst, and though remissions in petit mal have been produced by an insulin-glucose treatment which makes patients worse and then better,²³ only

the very rare patient seems to have any error in sugar metabolism which is the direct cause of seizures. This rarity is the patient with adenoma of the islets of Langerhans.

A person cannot realize the importance of the physiologic investigations of recent years unless his view includes their drab background. One element in this background was present in both ancient and modern times, the belief that seizures might come from a suprabiological source. The Freudian conception that the subconscious might throw an individual into a convulsion in order to simulate an intrauterine existence seems to be allied with the ancient conception of an indwelling demon doing his malignant daily dozen.

However, great as these contributions were, the investigation of epilepsy threatened to bog down. The influence of many physicochemical changes on seizures had been demonstrated, but it proved to be easier to start seizures than to stop them. Acidosis induced by a ketogenic diet in children, or by means of physical exercise, and possibly dehydration therapy, seemed to be the only measures of clinical value. None of the dozens of researches on body metabolism and blood chemistry had shown any consistent abnormality in epileptics (except perhaps an inconsistency and instability of many metabolic functions).²⁴ Various chemical changes would produce seizures, but only in those persons already subject to seizures. Many chemical changes could be demonstrated in the body and blood as a result of a convulsion but none as a precursor. Chemical alterations in the urine or venous blood of the arm bore an unknown relationship to changes in the metabolism of the brain. Convulsions induced in animals were a poor substitute for the spontaneous convulsions of patients. Even the century-old theory that a cerebral vascular spasm produced cerebral anemia and thus set off a seizure lost its appeal when a thermoelectric flow recorder placed in an internal jugular vein failed to record any decrease in cerebral flow before the onset of a seizure.²⁵ There seemed to be no avenue which would permit study of the activity of the living human brain and research came to a dead end.

In spite of the boasted receptivity of our age to new ideas, statements made by Berger of Jena in 1929²⁶ and thereafter that he could record the electrical potentials of the brain through the intact skull and that alterations of these potentials occurred during epileptic convulsions were ignored by physiologists and clinicians alike. After several years, however, repetition of his observations was attempted and his claim confirmed. With respect to epilepsy, the use of Berger's apparatus, the electroencephalograph, opened not just a new chapter but a new volume in the study and treatment of this disease. The interest of research workers has been revived. The clinicians, instead of guessing from the actions of the patients what is going on in the brain, can watch the brain itself at work. The patient, inured through the centuries to painful experimentation, can sit in an easy chair and let his brain write its own verdict on the diagnosis and prognosis of its disorder. The results of the studies of Gibbs, Gibbs, and Lennox,²⁷ which have been carried on during the past five years and which in some respects have been amplified by others, may be summarized as follows:

1. Seizures are accompanied by profound alterations in the rate and in the voltage of the electrical pulsations of the cortex. This is apparently true in all

eases except in those persons whose seizures are under conscious or subconscious control; hysterical seizures are not accompanied by dysrhythmia. Nevertheless, changes in brain activity consequent on attention or sensory stimulation (especially optic stimulation) and on sleep greatly alter the waves.

2. Approximately 95 per cent of patients subject to epileptic seizures show abnormalities in the electroencephalogram in a record taken for fifteen or twenty minutes when the patient is free of symptoms.

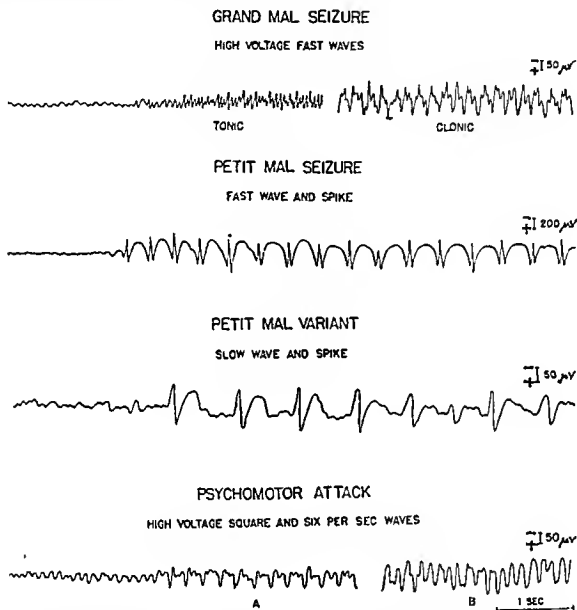


Fig. 1.—Patterns of electroencephalographic tracings encountered in patients with epilepsy during various types of seizures; namely, grand mal, petit mal, petit mal variant, and psychomotor (psychic equivalent) seizures. In the psychomotor attack, *A* represents the onset and *B* a later phase of the seizure. These abnormal patterns may appear either with or without seizures which are clinically observable. In each case the curve represents the difference in potential of the cortex between an electrode attached to the left forehead and one attached to the ear. The signal at the right indicates the deflection caused by 50 millivolts or, in the case of petit mal, 200 millivolts current. The time marked by one second is indicated in the right lower corner. (From Gibbs, Gibbs, and Lennox²¹)

3. The different types of seizures trace distinctive patterns on the electroencephalogram; namely, petit mal seizures are associated with alternately fast and slow waves recurring at the rate of 3 a second; a slower, 2 a second, rhythm forms a variant, which is of particular interest because, unlike the 3-a-second form, it is not easily influenced by changes in the chemistry of the blood. Psychic seizures are accompanied by slow, high voltage waves, with oftentimes a square, crenated top. Grand mal attacks are accompanied by fast, high potential waves (Fig. 1).

4. In many patients coming grand mal attacks can be predicted by the increase in fast waves in the electroencephalogram for as many as eighteen hours preceding the attack.

5. In many patients the abnormal waves begin in a certain area, or waves in this area are more abnormal than elsewhere. If this area should be a frontal lobe with the dysrhythmia limited to it, lobectomy might prove beneficial.

6. In case of a cortical lesion, such as one caused by a tumor or trauma, electroencephalographic examination discloses abnormal waves at the periphery of the lesion.²⁸⁻³¹ The procedure is, therefore, useful to the surgeon in localizing lesions (whether patients are epileptic or not) which might require surgical intervention.

7. The rhythm of the brain is apparently a constitutional characteristic, for in spite of the many conditions which may modify the electroencephalogram, an individual who has a distinctive pattern tends to show this same pattern, either normal or abnormal, when his record is taken under standard conditions on successive occasions. The normal tracings of healthy similar twins are closely similar,³² as are the abnormal tracings of patients;³³ and a large proportion (60 per cent) of the relatives of epileptics, both of the essential and symptomatic groups, have abnormal electroencephalographic records.³³ Approximately 10 per cent of persons without a history of epilepsy in their immediate families have cortical dysrhythmia. Since approximately 0.5 per cent of the population have a personal history of seizures, these data indicate that there are approximately 20 persons with a dysrhythmia to one who has seizures, or in the United States 10 million persons who have a predisposition to epilepsy, or a related disorder, to half a million actual epileptics. These observations raise very large personal and social questions concerning possible prophylaxis against epilepsy among predisposed individuals and in the race.

8. Although epilepsy can now be spoken of as a cerebral dysrhythmia, it is only one of many. Dysrhythmia may be asymptomatic or symptomatic. If symptomatic, the associated manifestations may take the form of seizures, or periods of misbehavior as in "problem children,"³⁴ or in mental disorders.³⁵⁻³⁸ The borderlands of clinical epilepsy are ill defined. Cerebral dysrhythmia includes many of these borderland conditions. In epilepsy Gibbs³⁹ believes that when there is cortical dysrhythmia the primary disturbance is in the chemio-electrical oscillators of the brain. If a chemical regulator of the brain can be found, many types of dysrhythmia associated with clinical disturbances which now bear various names can be helped.

As an aid in the interpretation of records, Grass⁴⁰ has utilized the principle of the Fourier transform to build an apparatus for the analysis of records by electrical means. This analyzer summates the total energy contained in the waves of various frequencies, the faster of which are not easily detected in the ordinary tracings, and it also allows accurate comparison of the records of various persons, with respect to the amount of energy present in the various wave frequencies.

9. The rate and voltage of waves can be altered by changes in blood chemistry. This fact permits a resumption of the promising chemical studies of a decade ago. The investigator no longer needs to wait for the patient to have a

convulsion. He can watch the progress of the disorder on the moving paper tape. Observations with the electroencephalograph have confirmed previous clinical observations, for alkalosis, anoxemia, and hypoglycemia increase dysrhythmia. However, a previously undreamed-of opening for study has appeared in the fact that certain chemical changes in the blood can be correlated with certain variations in the rate of brain waves, and these with various types of seizures. Artificial reduction of carbon dioxide in the alveolar air and arterial blood slows the frequency of brain waves and is especially potent in precipitating petit mal which has an extremely slow 3-a-second wave. Increasing the carbon dioxide, on the other hand, speeds up brain waves and inhibits petit mal. Furthermore, and this seems especially significant, patients who have only petit mal tend to have a low concentration of carbon dioxide in the arterial and internal jugular blood, whereas patients who have grand mal tend to have an abnormally high value.⁴¹ This statement refers to blood taken at odd times, but in a few observations which have been made arterial carbon dioxide seems to fall before a petit mal and to rise before a grand mal. The evidence is not yet complete, because it has not been shown that overventilation will inhibit a grand mal and high carbon dioxide will precipitate it.

Efforts are now being made to evaluate the various factors in the acid-base balance of blood passing through the brain in their relationship to seizures and to dysrhythmia. In these studies a lumbar puncture needle is placed in an internal jugular vein and in a femoral artery, and blood samples are withdrawn simultaneously from these two vessels before, during, and after seizures, and with relation to procedures which alter blood chemistry and brain waves. So far these observations indicate that small changes in carbon dioxide are much more influential in altering the electrical activity of the brain than are changes in oxygen or glucose. The changes in cerebral blood flow which follow changes in arterial carbon dioxide seem to be for the purpose of protecting the brain against abrupt fluctuations of carbon dioxide tension.⁴² Studies of the nomograms expressing the relationships of the carbon dioxide content and tension and the pH of arterial and internal jugular blood indicate distortions of the nomogram in epileptic patients as compared with nonepileptic persons,⁴³ and after artificial changes in carbon dioxide level an abnormally slow return of the cerebral circulation to its former flow.

Obviously these investigations are but a step toward an understanding of the physico-electrochemical processes which are concerned in the discharge, normal and abnormal, of the neurones of the brain. However, the gateway to new adventures in the realm of fundamental factors in the activity of nerve cells is now open for those who have the technical knowledge, equipment, and money to proceed.

These investigations are aimed primarily at an understanding of the underlying etiology of epilepsy, the peculiar inherited characteristics of neurones which cause them to discharge in a manner inimical to the welfare of the individual. Electroencephalographic examinations are of practical value to the physician and patient in judging the severity of the dysrhythmia and its response to treatment, in localizing cortical lesions when these are present, and in tracing the descent of epilepsy and related cerebral disorders. These newer

studies have answered many questions which have troubled students, but they have added others. For example, why do some persons with dysrhythmia have symptoms and others have none? Can the physician be content with stopping seizures but not dysrhythmia? What must be said to normal persons who have no suggestive family history of epilepsy or migraine but who do have dysrhythmia? Epilepsy research factories have a "backlog" of orders for many years to come.

Of newer aids to therapy, sodium diphenyl hydantoinate (dilantin sodium) is outstanding. Putnam and Merritt⁴⁴ used a method similar to one employed also by Spiegel⁴⁵ for producing convulsions in animals by passing an electric current through the brain. The amount of current required to produce a convulsion could be determined for an individual animal and the effect of the various drugs in preventing convulsions could be evaluated. Merritt and Putnam⁴⁶ tested scores of drugs, several of which seemed to have a higher anti-convulsant value than either of the accepted anticonvulsants, bromides, or phenobarbital. One of these, sodium diphenyl hydantoinate, was put to clinical tests and has proved its usefulness. Experience has shown that for the majority of patients dilantin sodium is more effective in controlling attacks than other drugs, and it has a relatively slight hypnotic effect. It is much more effective in stopping psychic and grand mal than in stopping petit mal seizures. More careful supervision of the patient and the dosage is required than for bromide or phenobarbital. The amount given must be increased until satisfactory therapeutic results are achieved, or until unpleasant side actions occur. This means that many patients given this treatment will show one or more of these side actions, namely, gastric irritation, ataxia, skin eruptions, or hyperplasia of the gums. Persons not sufficiently helped by dilantin sodium alone may be given phenobarbital or phenobarbital and benzedrine. For the large number of patients whom the drug dramatically helps, the year 1938 will be a milestone, just as 1857 (bromides) and 1912 (phenobarbital) have been milestones for previous generations of epileptics. Though not of such widespread usefulness, the demonstration by Cobb and Cohen⁴⁷ that certain dyes exert an inhibitory effect on seizures is of much theoretical interest. Aird⁴⁸ suggests that the dye may clog the blood spinal fluid barrier and thus prevent supposed convulsive toxins from entering the brain. Experiments with animals are handicapped by the fact that animals have not been used (if indeed any exists) which have an underlying cerebral dysrhythmia and spontaneous seizures. Animals made epileptic belong to the so-called symptomatic variety. Nevertheless, convulsions induced in unanesthetized animals by electric shock, either direct or from remote excitation,⁴⁹ or by convulsant drugs, permit evaluation of measures which affect seizure threshold, and offer a means for following the neurologic pathway of a seizure.⁵⁰ More nearly approximating clinical conditions are cerebral lesions which in the course of hours or days are followed by apparent spontaneously appearing seizures. This has been accomplished through freezing small areas of the cortex by Speransky⁵¹ and through very high roentgen-ray exposures by Derbyshire.⁵²

Advances have been made also in special fields. The operating room, especially that of Penfield,⁵³ has been used as a laboratory for tracing in the exposed cortices of patients "trigger zones" of Jacksonian seizures and for

observing spontaneous vascular responses to cortical stimulation as well as those which follow it. Careful histologic studies of the brain have demonstrated that an area of necrosis caused by a small vascular or other lesion may grow larger over a period of months or years, thus accounting for the long interval which may elapse between a head injury and the first seizure.⁵⁴ Also, bold surgery has freed many selected patients of seizures due to tumor or trauma.⁵⁵

Part of the progress of research in epilepsy has been the elimination of groups of persons who rightfully belong in some category besides epilepsy. Many a patient labeled "hysterical" or "psychopathic personality" or simply "mean," can now be diagnosed as dysrhythmic and treated as a sick person instead of an emotional or bad one. Rarely has the person labeled "epileptic" been proved hysterical. Through the work of Weiss and his associates,⁵⁶ the physician is now able to sift from his epileptics the occasional patient whose seizures are due to an irritable carotid sinus and whose symptoms can be relieved by atropine, benzedrine, or by extirpation of the sinus. The study of migraine, which is genetically related to epilepsy,⁵⁷ has shown the importance of abnormal relaxation of the branches of the external carotid artery in the production of headache⁵⁸ and the dramatic relief of individual attacks of migraine which can be obtained by the injection of ergotamine tartrate.⁵⁹ Persons with narcolepsy have also been vastly aided by drug treatment with benzedrine sulfate.⁶⁰

Scientific advances in the understanding and treatment of epilepsy in the last twenty years have been prodigious, but public appreciation of the size of the social and economic aspects of the problem, and correction of the popular attitude toward persons with seizures have lagged far behind. Popular ignorance, fear, and prejudice are reminiscent of the Middle Ages. Gradually the public must learn that epilepsy is not necessarily or usually associated with mental deterioration, and that most epileptics, if given a chance, can be useful citizens. The problem of the ten million potential epileptics should not arouse fear, but a determination to learn more about epilepsy and dysrhythmia and their treatment.

As an experiment in this direction an organization called the "Laymen's League Against Epilepsy" has been formed. Anyone may become a member and pay either \$1.00 or \$5.00 annually. Each member receives a series of bulletins which give authoritative information about the whole subject of epilepsy, and a portion of the dues are used in carrying on research work in this field. Among physicians the "American League Against Epilepsy" (to which any interested and qualified physician may belong) is a chapter of the international organization. In epilepsy there would seem to be unusual opportunity and need for medical investigators, medical practitioners, patients, and the general public to unite in trying to solve a problem which is a puzzle and a pain to all.

REFERENCES

1. Geyelin, H. R.: Fasting as a Method for Treating Epilepsy, *M. Rec.* 99: 1037, 1921.
2. Wilder, R. M.: The Effects of Ketonuria on the Course of Epilepsy, *Mayo Clin. Bull.* 2: 307, 1921.
3. Peterman, M. G.: Ketogenic Diet in the Treatment of Epilepsy, *Am. J. Dis. Child.* 28: 28, 1924.

4. Barborka, C. J.: Epilepsy in Adults: Results of Treatment by Ketogenic Diet, *Arch. Neurol. & Psychiat.* 23: 940, 1930.
5. Helmholz, H. F., and Keith, H. M.: Eight Years' Experience With the Ketogenic Diet in the Treatment of Epilepsy, *J. A. M. A.* 95: 707, 1930.
6. Helmholz, H. F., and Goldstein, M.: Results of 15 Years' Experience With Ketogenic Diet in the Treatment of Epilepsy in Children, *Am. J. Psychiat.* 94: 1205, 1938.
7. Talbot, F.: Treatment of Epilepsy, New York, 1930, Macmillan Co.
8. Wilkins, L.: Epilepsy in Childhood, *J. Pediat.* 10: 317, 1937.
9. Bigwood, E. J.: L'équilibre physico-chimique du sang dans l'épilepsie. L'ion calcium, *Ann. de méd.* 15: 24, 1924.
10. Lennox, W. G., and Cobb, S.: Epilepsy (Medicine Monographs, Vol. XIV), Baltimore, 1928, Williams & Wilkins Co.
11. Rosett, J.: The Experimental Production of Rigidity of Abnormal Involuntary Movements and of Abnormal States of Consciousness in Man, *Brain* 47: 293, 1924.
12. Foerster, O.: Hyperventilationsepilepsie, *Deutsche Ztschr. f. Nervenhe.* 83: 347, 1924-1925.
13. Lennox, W. G., and Gibbs, E. L.: The Blood Flow in the Brain and the Leg of Man, and the Changes Induced by Alteration of Blood Gases, *J. Clin. Investigation* 11: 1155, 1932.
14. Spiegel, E., and Spiegel-Adolf, M.: Physico-chemical Mechanisms in Convulsive Reactivity, *Proc. Soc. Exper. Biol. & Med.* 34: 799, 1936.
15. Lennox, W. G., and Behnke, A. R.: Effect of Increased Oxygen Pressure on the Seizures of Epilepsy, *Arch. Neurol. & Psychiat.* 35: 782, 1936.
16. Fay, Temple: Therapeutic Effect of Dehydration on Epileptic Patients, *Arch. Neurol. & Psychiat.* 23: 920, 1930.
17. Cobb, Stanley: Causes of Epilepsy, *Arch. Neurol. & Psychiat.* 27: 1245, 1932.
18. Fremont-Smith, F., and Merritt, H. H.: Influence of Variation in Fluid Intake on Intracranial Pressure in Epileptics, *Arch. Neurol. & Psychiat.* 29: 454, 1933.
19. McQuarrie, I., and Peeler, D. B.: Effects of Sustained Pituitary Antidiuresis and Forced Water Drinking in Epileptic Children, a Diagnostic and Etiologic Study, *J. Clin. Investigation* 10: 915, 1931.
20. Wortis, S. B.: Effects of Insulin, Metrazol, and Camphor Convulsions on Brain Metabolism, *New York State J. Med.* 38: 1015, 1938.
21. Gellhorn, E.: Effects of Hypoglycemia and Anoxia on the Central Nervous System. A Basis for a Rational Therapy of Schizophrenia, *Arch. Neurol. & Psychiat.* 40: 125, 1938.
22. Maddock, S., Hawkins, J. E., and Holmes, E.: The Inadequacy of Substances of the "Glucose Cycle" for Maintenance of Normal Cortical Potentials During Hypoglycemia Produced by Hepatectomy With Abdominal Evisceration, *Am. J. Physiol.* 125: 551, 1939.
23. Gibbs, F. A., Gibbs, E. L., and Lennox, W. G.: Influence of Blood Sugar Level on Wave and Spike Formation of Petit Mal Epilepsy, *Arch. Neurol. & Psychiat.* 4: 1111, 1939.
24. Goldstein, H., and McFarland, R. A.: The Biochemistry of Epilepsy. A Review, *Am. J. Psychiat.* 96: 771, 1940.
25. Gibbs, F. A., Gibbs, E. L., and Lennox, W. G.: Cerebral Blood Flow Preceding and Accompanying Epileptic Seizures in Man, *Arch. Neurol. & Psychiat.* 32: 257, 1934.
26. Berger, H.: Über das Elektroencephalogramm des Menschen, *Arch. f. Psychiat.* 87: 527, 1929.
27. Gibbs, F. A., Davis, H., and Lennox, W. G.: The Electro-encephalogram in Epilepsy and in Conditions of Impaired Consciousness, *Arch. Neurol. & Psychiat.* 34: 1133, 1935.
- Gibbs, F. A., Gibbs, E. L., and Lennox, W. G.: The Electro-encephalogram in Diagnosis and in Localization of Epileptic Seizures, *Arch. Neurol. & Psychiat.* 36: 1225, 1936.
- Lennox, W. G., Gibbs, F. A., and Gibbs, E. L.: Effect on the Electro-encephalogram of Drugs and Conditions Which Influence Seizures, *Arch. Neurol. & Psychiat.* 36: 1235, 1936.
- Gibbs, F. A., Gibbs, E. L., and Lennox, W. G.: Epilepsy: A Paroxysmal Cerebral Dysrhythmia, *Brain* 60: 377, 1937.
28. Walter, W. C.: The Location of Cerebral Tumors by Electroencephalography, *Lancet* 2: 305, 1936.
29. Williams, D., and Gibbs, F. A.: Localization of Intracranial Lesions by Electroencephalography, *New England J. Med.* 218: 998, 1938.
30. Case, T. J., and Bucy, P. C.: Localization of Cerebral Lesions by Electroencephalography, *J. Neurophysiol.* 1: 245, 1938.
31. Williams, D., and Gibbs, F. A.: Electroencephalography in Clinical Neurology, *Arch. Neurol. & Psychiat.* 41: 519, 1939.
32. Davis, H., and Davis, P. A.: Action Potentials of the Brain in Normal Persons and in Normal States of Cerebral Activity, *Arch. Neurol. & Psychiat.* 36: 1214, 1936.
33. Lennox, W. G., Gibbs, E. L., and Gibbs, F. A.: The Inheritance of Epilepsy as Revealed by the Electroencephalograph, *J. A. M. A.* 113: 1002, 1939; The Inheritance of Cerebral Dysrhythmia and Epilepsy, *Arch. Neurol. & Psychiat.* In press.

34. Jasper, H. H., and Nichols, I. C.: Electrical Signs of Cortical Function in Epilepsy and Allied Disorders, *Am. J. Psychiat.* 94: 835, 1938.
35. Hoagland, H.: Electroencephalograms in Schizophrenia, *Arch. Neurol. & Psychiat.* 39: 210, 1938.
36. Davis, P. A., and Davis, H.: The Electroencephalograms of Psychotic Patients, *Am. J. Psychiat.* 95: 1007, 1939.
37. Gibbs, F. A., Gibbs, E. L., and Lennox, W. G.: The Likeness of the Cortical Dysrhythmias of Schizophrenia and Psychomotor Epilepsy, *Am. J. Psychiat.* 95: 255, 1938.
38. Jasper, H. H., Fitzpatrick, C. P., and Solomon, P.: Analogies and Opposites in Schizophrenia and Epilepsy: Electroencephalographic and Clinical Studies, *Am. J. Psychiat.* 95: 835, 1939.
39. Gibbs, F. A., Gibbs, E. L., and Lennox, W. G.: Cerebral Dysrhythmias of Epilepsy: Measures for Their Control, *Arch. Neurol. & Psychiat.* 39: 298, 1938.
40. Grass, A. M., and Gibbs, F. A.: A Fourier Transform of the Electroencephalogram, *J. Neurophysiol.* 1: 521, 1938.
41. Gibbs, E. L., Lennox, W. G., and Gibbs, F. A.: Variations in the Carbon Dioxide Content of the Blood in Epilepsy, *Arch. Neurol. & Psychiat.* 43: 223, 1940.
42. Lennox, W. G., Gibbs, F. A., and Gibbs, E. L.: The Relationship in Man of Cerebral Activity to Blood Flow and to Blood Constituents, Baltimore, 1938, Williams & Wilkins Co., *A. Research Nerv. & Ment. Dis., Proc. Vol. XVIII*, page 277.
43. Nims, L. F., Gibbs, E. L., Lennox, W. G., Gibbs, F. A., and Williams, D.: Adjustment of Acid Base Balance of Patients With Petit Mal Epilepsy to Overventilation, *Arch. Neurol. & Psychiat.* 43: 262, 1940.
44. Putnam, T. J., and Merritt, H. H.: Experimental Determination of the Anticonvulsant Properties of Some Phenyl Derivatives, *Science* 85: 525, 1937.
45. Spiegel, E. A.: Procedures in Experimental Convulsions, *Tr. Am. Therap. Soc.* 37: 37, 1938.
46. Merritt, H. H., and Putnam, T. J.: Sodium Diphenyl Hydantoinate in the Treatment of Convulsive Disorders, *J. A. M. A.* 111: 1068, 1938.
47. Cobb, S., and Cohen, M. E.: Anticonvulsive Action of Vital Dyes, *Arch. Neurol. & Psychiat.* 40: 1156, 1938.
48. Aird, R. B.: Mode of Action of Brilliant Vital Red in Epilepsy, *Arch. Neurol. & Psychiat.* 42: 700, 1939.
49. Fender, F. A.: Epileptiform Convulsions From "Remote" Excitation, *Arch. Neurol. & Psychiat.* 38: 259, 1937.
50. Erickson, T. C.: Spread of the Epileptic Discharge, *Arch. Neurol. & Psychiat.* 43: 429, 1940.
51. Speransky, A.: La congélation des tissus; procédé d'obtention de l'autoneurotoxine et d'autres autotoxines cellulaires, *Ann. de l'Inst. Pasteur* 40: 213, 1926.
52. Derbyshire, A. J., and Corrigan, K. E.: Electroencephalographic Studies on Irradiated Animal Brains, *Am. J. Physiol.* 126: 476, 1939.
53. Penfield, W., and Boldrey, E.: Somatic Motor and Sensory Representation in the Cerebral Cortex of Man as Studied by Electrical Stimulation, *Brain* 60: 389, 1937.
54. Penfield, W., and Humphreys, S.: Epileptogenic Lesions of the Brain. A Histologic Study, *Arch. Neurol. & Psychiat.* 36: 449, 1936.
55. Penfield, W.: Epilepsy and Surgical Therapy, *Arch. Neurol. & Psychiat.* 43: 240, 1940.
56. Weiss, S., Capps, R. B., Ferris, E. B., and Munro, D.: Syncope and Convulsions Due to a Hyperactive Carotid Sinus Reflex: Diagnosis and Treatment, *Arch. Int. Med.* 58: 407, 1936.
57. Lennox, W. G.: Migraine and Epilepsy: Newer Concepts and Treatment, *J. Med.* 19: 284, 1938.
58. Graham, J. R., and Wolff, H. G.: The Mechanism of Migraine Headache and Action of Ergotamine Tartrate, Baltimore, 1938, Williams & Wilkins Co., *A. Research Nerv. & Ment. Dis., Proc. Vol. XVIII*, page 638.
59. Lennox, W. G.: The Use of Ergotamine Tartrate in Migraine, *New England J. Med.* 210: 1061, 1934.
60. Prinzmetal, M., and Bloomberg, W.: The Use of Benzedrine for the Treatment of Narcolepsy, *J. A. M. A.* 105: 2051, 1935.

THE DEVELOPING EGG AS A CULTURE MEDIUM*

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THIS paper deals with the chick embryo and its membranes as an individual living experimental host for a variety of infectious agents rather than as the source of viable cells and juices for tissue cultures.¹ The developing egg has proved to be susceptible to a greater number of viruses than any other living host, and it has also been found to be infectable by certain protozoa, spirochetes, fungi, bacteria, and rickettsiae.² To many of these infectious agents the hatched chick possesses an apparently complete natural resistance. The chick embryo, therefore, has a very high degree of susceptibility inherent in its cells and its developmental environment; it seems to have little or no capacity to acquire specific immunity before hatching.

The chick embryo is exceptionally useful for the study of many problems of infection of widely different kinds, and of resistance—natural, acquired, and passively transferred. Because of the large number and variety of infectious agents that can be cultivated in a pure state in the chick embryo, it is also a useful medium for the production in quantity of uncontaminated vaccines.

It has also been found that certain viruses can be modified by successive infections in embryos in such a way that they become less virulent for other hosts, so that in the modified form they can be used as infectious vaccines. Some viruses multiply enormously in the infected embryo in fully virulent form; this fact has facilitated the production of certain vaccines that can be used after inactivation.

Historically the first published account of experimental infection of the chick embryo of which I am aware was Levaditi's report, published in 1906, describing his results with the spirillum of fowls discovered by Marchoux and Salimbeni.³ Levaditi acknowledged his indebtedness for the technique to Borrel who infected embryos the year before with this microorganism but did not publish his observations.

Levaditi's experiments were of particular significance in that they suggested a relationship between the life processes of the embryo and the susceptibility to spirillosis. They emphasized also the phenomenon of the passive transfer of immune bodies through the egg from the mother to the offspring.

Rous and Murphy⁴ published in 1911 their results of implanting and inoculating chick embryos with a transmissible sarcoma of fowls and its filtrate. These experiments had important bearing upon virus infections and tumor culture, and upon matters relating to organic differentiation as they might affect susceptibility and immunity.

In 1920 Juan and Staub⁵ infected chick embryos with the virus of avian pest, and in 1929 Gay and Thompson⁶ obtained evidence indicating the propaga-

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tion of vaccinia virus in these animals. Woodruff and Goodpasture,⁷ and Woodruff, Goodpasture, and Buddingh,⁸ demonstrated in 1931 the susceptibility of the chorio-allantois of chick embryos to the viruses of fowl pox, herpes simplex, and vaccinia. Their investigations called attention to the unusual susceptibility of chick embryonic tissues to viruses and to the possibilities inherent in the method for the study of a variety of infectious agents. Subsequently, Goodpasture and Anderson,⁹ Gallavan and Goodpasture,¹⁰ and Buddingh and Polk¹¹ demonstrated clearly the usefulness of the chick embryo for studies of the pathogenesis of bacterial infections, including those of *Hemophilus pertussis*, *Hemophilus influenzae*, and *Neisseria meningitidis*, for which there is no readily available host other than man.

Following these studies the chick embryo has been used extensively and the method has become recognized as a useful procedure for the investigation of virus infections and those infections due to a variety of parasites.^{9, 12}

METHOD

It is necessary to have a constant source of fertile eggs. The poultry industry has made available excellent types of incubators which are relatively inexpensive. The best ones are electrically operated and are provided with automatic control of heat and moisture and a simple device for daily rotation of the eggs.

As a rule, the younger the embryos the worse they stand operative procedures. For this reason, we prefer to use, wherever possible, embryos that have incubated for ten to fourteen days. The normal incubation period for hen eggs is twenty-one days; consequently, several days are available for experimental observations.

Opening the eggs, at first a tedious procedure, has been made simple, rapid, and uniform by the use of a cutting carborundum disk, rotated by means of a dental motor with attached flexible shaft and chuck. This machine is operated by means of a foot treadle which permits variable speed. Underlying the egg shell is a tough, fibrous membrane, called the shell membrane, which is a secretion product of the isthmus of the hen's uterus. When cutting a window in the shell for the purpose of inoculation or observation, the calcinated shell alone is first penetrated by means of the carborundum disk. The area outlined by the cut is then covered with a thin coat of hot melted paraffin to fix loose particles and to prevent contamination during succeeding operations. Next, the fibrous membrane is penetrated with a spear point needle and cut along the shell incision, leaving one side of the cap for a hinge or, if the cap is to be discarded, that side is easily torn across by pulling away the shell cap.¹³

Only eggs that have been proved to contain living embryos are operated upon. Fertility is determined by candling the egg; by this means the extent of development of the chorio-allantoic membrane may be seen, and its large blood vessels located if it is desired to expose them for purposes of injection or otherwise.

Beneath the fibrous membrane, upon which the calcium is deposited with its coating of mucons, lies the chorio-allantois, by means of which respiration takes place; on its outer surface is a layer of ectodermal epithelial cells inter-

rupted by abundant capillaries which underlie the epithelial layer and protrude through it. Beneath the epithelium, and supporting the blood vessels, is the fibrous tissue of the mesoderm, and below this is a continuous layer of endodermal epithelium. The chorio-allantois membrane is a very useful site to inoculate for many purposes; it can be viewed and manipulated easily and at will. The window, after inoculation or other operations, may be sealed by surrounding it with a layer of vaseline-paraffin mixture upon which is placed a cover glass. Through this cover glass one can see at any time what is going on superficially, and the membrane can be viewed through a dissecting microscope or, for higher magnification, through an Ultropaque equipment. If moisture collects beneath the glass, it can be readily removed by applying a blunt hot instrument to the glass cover.

Through the shell opening inoculations can be made into different parts of the embryo itself, into the amnion, or intravenously. Also, material can be removed at will for examination or culture. After the egg is inoculated, it is placed in an ordinary bacteriologic incubator on a wire tray with a diamond-shaped wire mesh of a size satisfactory to hold it in position with the window uppermost. The incubator temperature is maintained at about 37° C. For some purposes lower temperatures are advisable. For example, Bengtson and Dyer¹⁴ found that the rickettsiae of Rocky Mountain spotted fever were more numerous in inoculated eggs incubated at 33° C.

VIRUS INFECTIONS

The cells of the chick embryo have been found to be susceptible to infection by a much greater number of viruses than any other single host. Consequently, the embryo may be used as a convenient living medium for the cultivation and experimental investigation of a great variety of these agents.^{15, 16}

The following viruses that are pathogenic for man have already been cultivated in this medium, namely, those of smallpox, alastrim, and vaccinia; herpes simplex, virus B, yellow fever, and Rift Valley fever; St. Louis encephalitis, lymphocytic choriomeningitis, equine encephalomyelitis, louping ill of sheep, and rabies¹⁷; the common cold, epidemic influenza, and psittacosis.

Of viruses affecting animals, but to which man is apparently insusceptible, the following have been cultivated in chick embryos: Rous sarcoma, fowl pox, fowl plague, infectious laryngotracheitis of fowls, New Castle disease, infectious bronchitis of chickens, Pacheco's parrot disease, vesicular stomatitis of horses, myxomatosis of rabbits, ectromelia of mice, sheep pox, and pseudorabies.

Thus far among viruses affecting man, the following have resisted cultivation in the chick embryo, namely, those of molluscum contagiosum, warts, mumps, herpes zoster, varicella, poliomyelitis, and measles.

It has been reported that the virus of measles, for which there is no other adequately proved susceptible host than man, has been cultivated in the chick embryo, but there is as yet no acceptable confirmation.¹⁸

Most of the reports of virus infections have concerned only lesions as manifested in the chorio-allantois, little or no attention having been devoted either to distribution of virus in the embryo proper or to evidences of localization in

the body of the embryo as manifested by specific lesions. Frequently there has been no cytologic description of the membranal lesions themselves. Various routes of inoculating the embryo are accessible, and infection of the embryo proper, directly or by extension, offers great possibilities as yet too little realized. Classical examples of this potentiality are illustrated by the studies of vaccinia by Buddingh¹⁹ and of herpes simplex by Anderson.²⁰

It is to be emphasized that an adequate study of a virus infection, and for that matter any other sort of infection, should be founded upon a cytologic and histologic basis; and if one is to derive the most from the chick embryo method, morphology must direct investigations of pathogenesis. It is well known that most viruses induce characteristic changes in the cells they affect, and one can determine the course, distribution, and the fact of localized infections so well in no other way as by microscopic study of the cellular structure of the host.

RICKETTSIAL INFECTION

The rickettsiae of Mexican and European typhus fever have been cultivated in the chorio-allantois of chick embryos by Zia,²¹ and those of Rocky Mountain spotted fever by Bengston and Dyer.¹⁴ Cox has succeeded in growing the rickettsiae of Rocky Mountain spotted fever and of the typhus group in the yolk sac of the developing egg.²²

Inoculation of the chorio-allantois results in infection of the ectodermal epithelial cells, and it seems likely that growth in the yolk sac is the result of infection of the endodermal lining cells. No adequate study has been made of infection of the embryo proper by various routes of inoculation.

BACTERIAL INFECTION

The usefulness of the chick embryo method for the study of bacterial infection was indicated by Goodpasture and Anderson, who induced infections with pure cultures of *Staph. aureus*, *Strep. hemolyticus*, *Strep. viridans*, *B. aerogenes*, *E. typhi*, *B. abortus*, and *C. diphtheriae*. These authors called attention to intracellular growth of some of these strains and pointed out the importance of this phenomenon in the processes of invasion.⁹ Internal lesions of the embryo following membranal infection were described in some instances.

Subsequently, Gallavan,²³ and Gallavan and Goodpasture,¹⁰ described infection of the embryo with *H. influenzae* and *H. pertussis*. Localization of infection with the former microorganism in the brain and meninges and on the ciliated border of respiratory epithelium with the latter was described, and the similarity of these lesions to those encountered in human infections with the same bacteria was emphasized. Buddingh and Polk were able to infect embryos with the meningococcus, and under suitable conditions they reproduced, not only the septicemic form of disease, but also a specific suppurative meningitis.¹¹ Their results indicated that meningitis results from a localization of blood-borne meningococci.

Morrow and Berry²⁴ infected the chorio-allantois with *N. gonorrhoeae* and used this technique to study the effect of sulfanilamide therapy. This work was confirmed as regards infection by Hill and Pitts.²⁵

Other reports of bacterial infection of various kinds have been made by Pandit, Rao, and Shortt²⁶; Castil and Bloch²⁷; Mettzer, Beaudette, and Stokes²⁸; and Weil and Valentine.²⁹

SPIROCHETAL INFECTION

Morrow, Syverton, Stiles, and Berry³⁰ reported growth of *L. icterohaemorrhagiae* in the chick embryo, and this was confirmed by Davis.³¹ Chabaud³² and Oag³³ succeeded in infecting the embryo with *S. duttoni*. As yet there is no satisfactory evidence that *S. pallida* has been cultivated in this medium.

FUNGUS INFECTIONS

Goodpasture³⁴ suggested that the chick embryo method might be useful for study of fungus infections after observing the effect of certain contaminants on the chorio-allantois. Recently Moore³⁵ has called attention to the usefulness of the chick embryo for the study of a number of pathogenic fungi.

PROTOZOAL INFECTION

Levine, Brandly, and Graham³⁶ have infected chick embryos with *Tritrichomonas foetus* and Chabaud has used these embryos for studies of infection by certain pathogenic trypanosomes.³⁷

This incomplete list of publications will serve to indicate the usefulness of the chick embryo method for studies of a wide variety of infectious agents. Goodpasture, Douglas, and Anderson³⁸ have demonstrated the possibility of studying infection more indirectly by inoculating iso- and hetero-grafts of skin from susceptible animals, and Polk, Buddingh, and Goodpasture,³⁹ and Goodpasture and Anderson,⁴⁰ among others, have illustrated possibilities of the method for studies of immunity.

FUTURE POSSIBILITIES

The chick embryo is a living host that is susceptible to infection by a wide variety of pathogenic agents, and the possibilities of its use for study of pathogenesis are almost unlimited. Because of the practicability of introducing effective amounts of antiserum into the embryo, and because this immature animal apparently is incapable of producing antibodies in demonstrable quantity itself, an opportunity is afforded for studies of passive immunization.

Although the use of the chick embryo technique for investigation of chemotherapeutic agents of various kinds has only begun, it seems evident that it will afford an exceptionally good opportunity for chemotherapeutic experimentation.

The usefulness of the method for the production of certain vaccines in quantity for administration either as infectious or as noninfectious antigens has already been demonstrated, and it would seem likely that its adaptability to this purpose will be greatly extended in the future.

Buddingh and I¹³ in recent years have developed a satisfactory method for preparing antismallpox vaccine in quantity, free of bacteria, by infecting the chorio-allantois of chick embryos. Modifications of this method have been used by others for the same purpose and with satisfactory results. Our own experience has led us to believe that a vaccine thus prepared could with ad-

vantage supplant calf lymph for human antismallpox prophylaxis. A recent extensive report by Stevenson and Butler⁴¹ from the National Lymph Establishment is very favorable to its use. The method described by Buddingh⁴² of preserving vaccine in sterile serum, not possible with contaminated lymph of the calf, greatly prolongs its activity even against the adverse effects of body temperature.

Assuming a role of immense importance in individual and mass protection against yellow fever, endemic on two continents, are the recently developed methods of vaccination by the use of attenuated strains of yellow fever virus. The strain used by the International Health Division of the Rockefeller Foundation, with which over a million persons have been vaccinated in South America, is propagated routinely in tissue cultures, but for vaccine production it is inoculated into chick embryos. The infected embryos yield an abundance of virus. The vaccine induces a mild infection, without significant clinical symptoms, followed by the appearance of antiviral antibodies in the circulating blood signifying protective immunity.

Among the newcomers of viruses discovered to be pathogenic for man is that of equine encephalomyelitis, now thought to be an endemic infection among a variety of birds rather than a natural disease of horses. There are two immunologically distinct strains of this virus in our country, namely, the Eastern and the Western strains, and during the past year fatal infection with each has occurred in human beings. Both are highly infectious for chick embryos.

Beard and his co-workers⁴³ have described a vaccine prepared from virus cultivated in the chick embryo and treated with formalin that is being widely used with apparent success in animal prophylaxis. It is to be hoped that human infection with this virus will not reach proportions requiring similar immunizing procedures.

Beaudette⁴⁵ states that the New Jersey College of Agriculture supervises the production of laryngotracheitis vaccine at the Vineland Poultry Laboratories to insure purity and potency of the product. This virus is grown on the chorio-allantois with excellent results. These laboratories also produce their pigeon and fowlpox vaccine in the same way, i.e., by cultivation in the chick embryos.

Several publications have demonstrated the usefulness of the chick embryo method for inducing variation of certain viruses in the direction of lessened virulence thereby affording the possibility of experimental development of strains suitable for prophylactic vaccination. Thus far this has been accomplished, as indicated by the reports of Burnet⁴⁴ with the virus of influenza; of Beaudette⁴⁵ with the virus of bronchitis of fowls; of Penna and Mousatche⁴⁶ with the virus of yellow fever; of Buddingh⁴⁷ with the virus of fowlpox; of Anderson²⁰ with the virus of herpes simplex; and recently, of Dawson⁴⁸ with the virus of rabies. The possibility of future developments along this line for modifying not only viruses but also other types of pathogenic agents would seem to be particularly promising.

Finally, there remains the probability that the chick embryo will eventually prove to be the means of cultivation of certain viruses that so far have resisted transmission to species other than those in which they occur naturally.

There are already indications that the virus of measles will infect chick embryos,¹⁸ and if this is confirmed, an opportunity might be afforded to produce an effective vaccine for human prophylaxis.

Possibly fruitful lines for investigation in relation to infection could be initiated by means of the chick embryo technique in the direction of attempts to influence susceptibility and resistance by experimental variation in vitamin content and hormonal balance. The problem of the nature of natural resistance can also be attacked, because in many instances the highly susceptible cells and tissues of the embryo become completely insusceptible with advancing maturity.

The potentialities of the chick embryo method for studies of infection have only begun to be recognized, and one may look forward hopefully to a much greater realization of its usefulness in the future.

REFERENCES

1. Goodpasture, E. W.: Some Uses of the Chick Embryo for the Study of Infection and Immunity, *Am. J. Hyg.* 28: 111, 1938.
2. Goodpasture, E. W.: Viral Infection of the Chick Embryo, *Ann. Int. Med.* 13: 1, 1939.
3. Levaditi, C.: La spirillose des embryons de poulet, *Ann. Inst. Pasteur* 20: 924, 1906.
4. Rous, P., and Murphy, J. B.: Tumor Implantations in the Developing Embryo. Experiments With a Transmissible Sarcoma of the Fowl, *J. A. M. A.* 56: 741, 1911.
5. Juan, C., and Staub, A.: Etude sur la peste aviaire, *Ann. Inst. Pasteur* 34: 343, 1920.
6. Gay, F. P., and Thompson, R.: Attempts to Cultivate Vaccine Virus in the Growing Chick Embryo, *Proc. Soc. Exper. Biol. & Med.* 26: 556, 1929.
7. Woodruff, A. M., and Goodpasture, E. W.: The Susceptibility of the Chorio-allantoic Membrane of Chick Embryos to Infection With the Fowl-pox Virus, *Am. J. Path.* 7: 209, 1931.
8. Goodpasture, E. W., Woodruff, A. M., and Buddingh, G. J.: The Cultivation of Vaccine and Other Viruses in the Chorio-allantoic Membrane of Chick Embryos, *Science* 74: 371, 1931.
9. Goodpasture, E. W., and Anderson, K.: The Problem of Infection as Presented by Bacterial Invasion of the Chorio-allantoic Membrane of Chick Embryos, *Am. J. Path.* 13: 149, 1937.
10. Gallavan, M., and Goodpasture, E. W.: Infection of Chick Embryos With *H. pertussis* Reproducing Pulmonary Lesions of Whooping Cough, *Am. J. Path.* 13: 927, 1937.
11. Buddingh, G. J., and Polk, A. D.: Experimental Meningococcus Infection of the Chick Embryo, *J. Exper. Med.* 70: 485, 1939.
12. Burnet, F. M.: The Use of the Developing Egg in Virus Research, Medical Research Council Report No. 220, London, 1936.
13. Goodpasture, E. W., and Buddingh, G. J.: The Preparation of Antismallpox Vaccine by Culture of the Virus in the Chorio-allantoic Membrane of Chick Embryos, and Its Use in Human Immunization, *Am. J. Hyg.* 21: 319, 1935.
14. Bengtson, I. A., and Dyer, R. E.: Cultivation of the Virus of Rocky Mountain Spotted Fever in the Developing Chick Embryo, *Pub. Health Rep.* 50: 1489, 1935.
15. Burnet, F. M.: The Growth of Viruses on the Chorio-allantois of the Chick Embryo, *Handb. d. Virusforschung*, R. Doerr and C. Hallauer, Wien, 1938, p. 419.
16. Sanders, M.: Cultivation of Viruses. A Critical Review, *Arch. Path.* 28: 541, 1939.
17. Dawson, J. R., Jr.: Infection of Chicks and Chick Embryos With Rabies, *Science* 89: 300, 1939; also personal communication.
18. Rake, G., and Shaffer, M. F.: Studies on Measles. I. The Use of the Chorio-allantois of the Developing Chick Embryo, *J. Immunol.* 38: 177, 1940.
19. Buddingh, G. J.: A Study of Generalized Vaccinia in the Chick Embryo, *J. Exper. Med.* 63: 227, 1936.
20. Anderson, K.: Pathogenesis of Herpes Simplex Virus Infection in Chick Embryos, *Am. J. Path.* 16: 137, 1940.
21. Zia, S.: The Cultivation of Mexican and European Typhus Rickettsiae in the Chorio-allantoic Membrane of the Chick Embryo, *Am. J. Path.* 10: 211, 1934.
22. Cox, H. P.: Use of Yolk Sac of Developing Chick Embryo as Medium for Growing Rickettsiae of Rocky Mountain Spotted Fever and Typhus Group, *Pub. Health Rep.* 53: 2241, 1938.
23. Gallavan, M.: Encephalitis and Meningitis in the Chick Embryo Following Inoculation of the Chorio-allantoic Membrane With *H. influenzae*, *Am. J. Path.* 13: 911, 1937.

24. Morrow, G., and Berry, G. P.: The Cultivation of *Neisseria Gonorrhoeae* on the Chorio-allantoic Membrane of Chick Embryos and the Use of This Technique for the Study of Sulfanilamide, *J. Bact.* 36: 280, 1938.
25. Hill, J. H., and Pitts, A. C.: The Growth of *Neisseria gonorrhoeae* on the Chorio-allantoic Membrane of the Chick Embryo, *J. Urol.* 41: 81, 1939.
26. Pandit, C. G., Rao, R. S., and Shortt, H. E.: Response of the Chorio-allantoic Membrane of the Developing Chick Embryo to Inoculation With Various Substances With Special Reference to *B. proteus* X-19, *Indian J. M. Research* 25: 595, 1938.
27. Castil, L., and Bloch, F.: Reactions de la membrane chorio-allantoïde de l'embryon de poulet aux bacillus tuberculeux humains et aviaires, *Compt. rend. Soc. de biol.* 128: 40, 1938.
28. Metzger, H. J., Beaudette, F. R., and Stokes, F. R.: Use of the Incubating Egg as a Differential Medium, *J. Am. Vet. M. A.* 95: 158, 1939.
29. Weil, A. J., and Valentine, J. A.: Infection of the Developing Chick Embryo With Dysentery Bacilli, *Proc. Soc. Exper. Biol. & Med.* 44: 160, 1940.
30. Morrow, G., Syverton, J. T., Stiles, W. W., and Berry, G. P.: The Growth of *Leptospira icterohaemorrhagiae* on the Chorio-allantoic Membrane of the Chick Embryo, *Science* 88: 384, 1938.
31. Davis, L. J.: The Susceptibility of the Developing Chick Embryo to *Leptospira icterohaemorrhagiae*, *Tr. Roy. Soc. Trop. Med. Hyg.* 33: 263, 1939.
32. Chabaud, A.: Infection de l'embryon de poule par *spirochaeta duttoni* et *spirochaeta icterohaemorrhagiae*, *Bull. Soc. path. exot.* 32: 483, 1939.
33. Oag, R. K.: The Growth of *Borrelia duttoni* in the Developing Egg, *J. Path. & Bact.* 49: 339, 1939.
34. Goodpasture, E. W.: Use of Chick Embryo in Investigation of Certain Pathological Problems, *South. M. J.* 26: 418, 1933.
35. Moore, M.: The Chorio-allantoic Membrane of the Developing Chick as a Medium for the Cultivation and Histopathologic Study of Pathogenic Fungi, *Science* 89: 514, 1939.
36. Levine, N. D., Brandly, C. A., and Graham, R.: The Cultivation of *Tritrichomonas foetus* in Developing Chicken Eggs, *Science* 89: 161, 1939.
37. Chabaud, A.: Infection de l'embryon de poule par quelques trypanosomes pathogenes, *Bull. Soc. path. exot.* 32: 489, 1939.
38. Goodpasture, E. W., Douglas, B., and Anderson, K.: A Study of Human Skin Grafted Upon the Chorio-allantois of Chick Embryos, *J. Exper. Med.* 68: 891, 1938.
39. Polk, A., Buddingh, G. J., and Goodpasture, E. W.: An Experimental Study of Complement and Hemolytic Amboceptor Introduced Into Chick Embryos, *Am. J. Path.* 14: 71, 1938.
40. Goodpasture, E. W., and Anderson, K.: Immunity to Fowl-pox Studied by Means of Skin Grafts on Chorio-allantois of Chick Embryo, *Arch. Path.* 30: 212, 1940.
41. Stevenson, W. D. H., and Butler, G. G.: Studies on the Cultivation of Vaccine on the Chorio-allantoic Membranes of Chick Embryos, Report on Public Health and Medical Subjects. No. 87. Ministry of Health, London, 1939.
42. Buddingh, G. J.: The Use of Serum as a Protective Diluent for Vaccinia Virus, *Am. J. Hyg.* 27: 530, 1934.
43. Beard, J. W., Finkelstein, H., Sealy, W. C., and Wyckoff, R. W. G.: Immunization Against Equine Encephalomyelitis With Chick Embryo Vaccine, *Science* 87: 490, 1938.
44. Burnet, F. M.: Influenza Virus on the Developing Egg. I. Changes Associated With an Egg-passage Strain of the Virus, *Brit. J. Exper. Path.* 17: 282, 1937.
45. Beaudette, F. R.: Personal communication.
46. Penna, H. A., and Moussatche, H.: Modification of Yellow Fever Virus by Serial Passages in the Developing Chick Embryo, *Brasil-med.* 38: 903, 1939.
47. Buddingh, G. J.: A Study of the Behavior of Fowl-pox Virus Modified by Intracerebral Passage, *J. Exper. Med.* 67: 933, 1938.
48. Dawson, J. R., Jr.: Personal communication.

THE VIRUSES*

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AS WE proceed down the scale of plant and animal life, from the largest to the smallest forms, no marked difficulties in differentiation are encountered until dimensions of 0.1 to 0.2 mm. are reached. At this level the hyphae of some of the molds and the itch mite are still clearly discernible to those with good vision. The wiggling extremities of the latter leave no doubt in the minds of the afflicted regarding classification. Continuing, one then enters the realm of the microcosm where with other types the yeasts and protozoa are unicellular representatives of the two great kingdoms. Near the lower border of the microscopie world stand the pleuropneumonia organisms, the Rickettsia and certain inclusion bodies associated with virus diseases, the morphology and structure of which are not too clearly revealed by the best staining methods. Are these forms plants or animals? Do the limits of microscopic vision coincide with an abrupt and exact transition point between the living and the non-living? The fundamental methods and techniques for the investigation of this problem have been mere extensions and refinements of the procedure employed with the bacteria; hence, progress to a marked degree has paralleled developments in this field.

The early acceptance of bacteria as agents of disease was based on the regularity with which they were present in the tissues, their growth on artificial media, and the infectivity of the cultures for susceptible animals. Detection, because of size, depended upon the microscope. The capabilities of this instrument were held to be entirely adequate to reveal all living things. Pasteur was the first to question this belief. In 1884 he found that the spinal cord of dogs which died of spontaneous rabies contained no visible microbes and gave no cultures in vitro, but embodied elements which multiplied when introduced subdurally into normal dogs or rabbits. With his usual genius and imagination Pasteur postulated the existence of a living submicroscopic world containing the same wealth of members as made up the microcosm. On this basis, the newly discovered rabies virus was the first representative. Thereupon, the term virus connoted a viable microscopically invisible agent rather than a poison. This interpretation of the word was promptly accepted, due in no small measure to the support of Pasteur and of additional outstanding discoveries.

The next development was in 1892 when, according to Iwanowski, the juices of plants with mosaic disease remained infectious after passage through the recently developed Chamberland filters of unglazed porcelain impervious to bacteria. Beijerinck confirmed and extended this observation, and in 1897 the infectious agent of hoof and mouth disease was recognized in what appeared

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to be sterile filtrates of diseased tissue. The designation, *virus*, now acquired additional meaning because of the filtration experiments, and it was applied for a long period of time only to those disease-producing entities, so-called filtrable viruses, capable of passing filters that retained ordinary bacteria. The agents of mosaic disease of plants, hoof and mouth disease, and on discovery, canine distemper, equine encephalomyelitis, lymphopathia venereum, poliomyelitis, influenza, yellow fever, and others, came under this category. In most instances, before the virus etiology of a disease could be established, it was necessary to account for and to eliminate the claims of certain visible organisms, either bacteria or protozoa, to this role. By way of example, there were the *B. cholerae* suis in hog cholera, *Bacillus X*, *B. icteroides*, and *Leptospira icteroides* in yellow fever, and more recently, greening streptococci in influenza and poliomyelitis.

During the past decade, following a renewed interest in viruses, and, as an outgrowth of intensive studies of their chemical and physical properties, the definition has been further narrowed with emphasis not only on their ability to traverse fine membranes, but also on the intimate relationship which seems to exist between virus and host cell, such as their intracellular position and their failure to grow in the absence of living cells. The viruses form a continuous series, ranging in size from within the lower limits of ordinary microscopic vision (vaccinia 250 $m\mu$) to dimensions of 10 $m\mu$ (hoof and mouth virus). In this submicrocosm, problems of classification and questions such as whether certain species are plants or animals assume only academic significance, they, the viruses, are unequivocally entitled to the designation, protists or first forms. The smallest viruses are theoretically beyond the range of the recently introduced electron microscope, and instruments employing shorter wave lengths or some other principle will have to be developed before we can make direct observations of the morphology of these agents which approach molecular dimensions. The available data have been ascertained by selective filtration, using graded collodion filters, and have been confirmed and extended by ultracentrifugation. In keeping with variation in the size of the cells of any one bacterial species, the viruses differ in size within quite definite limits.

Viruses were first recognized and described in terms of biological activity; they continue to be revealed only by means of this attribute. One of the most fruitful fields for the obtaining of fundamental information in virus research is with the plant pathogens of which there are many. The restrictions, such as excessive costs for the purchase and care of animals, elaborate quarters, and possibilities of human infection which confront those studying the animal viruses, are nonexistent. Here, one may have on hand at any one time thousands of infected plants in modest surroundings, and with no danger to those associated with the investigation. A few of the intriguing problems of viruses and virus infections, the unraveling of which at present seemingly offers insurmountable obstacles, will now be considered.

Viruses multiply only within certain living cells, thus their purposeful cultivation in vitro is limited to those media containing living tissue. Sedulous attempts to grow viruses in the absence of viable cells have failed, yet the belief that viruses are living agents depends upon the fact that they are capable of re-

production and adaptation in the living plant or animal body. When an animal, chick embryo, or tissue culture, such as the agar roll tube, is inoculated with a definite quantity of the virus, it is found, after a suitable incubation period, that the virus has reproduced itself in relatively enormous quantities, a small fragment of tissue yielding millions of infective doses. Perpetuation of the virus and its proliferation can be carried forward at will for unnumbered generations or passages. But why this intimate relationship between parasite and host cell? Two theories may give some insight into the phenomenon: (1) Is it a protective mechanism from toxic substances in the extracellular spaces? or (2) May we assume that a virus is an organism which by persistent localization within the cell is deficient in enzyme systems and has thus lost the power to synthesize its own nutrient materials to the state at which they are active within the cell; and they, therefore, cannot grow in extracts of the cell but only within the cell itself? If we follow this latter theory to its logical conclusion, we may deduce the nonviable nature of viruses, which attitude at present is decidedly popular and is gaining in favor. It is necessary merely to postulate that in this adaptation to an intracellular existence the enzymes have been progressively lost and appropriated from the cell. Ultimately a mere nucleoprotein is left which is itself inert and only exhibits the characters of life, borrowing and directing the enzyme system from the cell it invades for its own reproduction. Such an implication is in keeping with the work of Stanley, who has isolated the virus of tobacco mosaic, as well as certain other viruses, as crystalline nucleoproteins with definite chemical and physical properties. Nevertheless, it should follow that if this process is essentially progressive, viruses should exist which have not yet lost their power of synthesis to such an extent that an intracellular existence is mandatory. The ubiquitous distribution of saprophytic viruses is predicated by certain British workers who have actually isolated several species from sewage and grown them in cell free media. If we adopt the first theory, which accepts viruses as living entities, the attainment of the goal of the *in vitro* cultivation resolves itself into one of two possible plans: (1) the development of techniques for the neutralization or removal of toxic substances, or (2) the accurate reproduction within the culture tube of the nutritive conditions within the cell, thus permitting the virus to grow in a sterile medium devoid of cells. The second concept becomes a straightforward problem of analytical and synthetic organic chemistry, but is tinged by the evidence from epidemiologic observations that a belief in the spontaneous fabrication of viruses by host cells is unwarranted.

Many virus-infected cells contain inclusion bodies appearing in the cell cytoplasm or nucleus, or in both cytoplasm and nucleus. On rare occasions the inclusion bodies characteristic of two different virus diseases may be present simultaneously in the same cell, thus evidence of a double infection. The inclusion bodies are pleomorphic, of various kinds and peculiar to certain types of cells. Negri bodies discernible within the ganglion cells of the brains of animals affected with spontaneously occurring rabies, and Guarnieri bodies within the epidermal cells in lesions of vaccinia are among the best known

examples. They stain readily and are easily recognizable in clinical material, yet the nature, significance, and relationship of these and other inclusion bodies to the specific virus with which they are associated are not clear.

In diagnostic procedures the presence or absence of inclusion bodies is of considerable importance. Unfortunately, their distribution is limited to a relatively small proportion of the virus diseases. Are the inclusion bodies and the virus one and the same, the former aggregates of the latter? Are the inclusion bodies degenerate products of cells attacked by virus, or are they virus clothed with cellular material? There are sponsors for each of the three theories. Preparations of pure inclusion bodies, the elementary bodies of vaccinia, have been obtained and subjected to biological studies. It appears from the results that the elementary body is a viable organism deficient in certain enzymes.

The viruses, like bacteria, manifest a striking selective affinity for particular tissues in the bodies of their host, as for example, the neurotropic viruses of rabies, lethargic encephalitis, poliomyelitis, and equine encephalomyelitis, and dermatropic herpes virus. The viruses in these diseases presumably proliferate only in the tissues attacked according to their selective affinities. A number of virus diseases occur as acute, generalized infections, notably among which are hog cholera, distemper, and influenza. In these the causative viruses multiply in the circulating blood and viscera and, as might be expected, are concentrated in these locations.

A striking feature of many of the viruses, which is not highly developed among bacteria, is their adaptation to foreign hosts and individual tissues. Such virus agents as poliomyelitis can be ultimately perpetuated in certain species of monkeys, and vaccinia, yellow fever, and herpes, which are not neurotropic in natural infections, may be adapted to the nervous system of laboratory animals by experimental procedures. Considerable difficulty may be encountered in making the initial transfer from the natural to the experimental host. The first "take" may cause little disturbance or escape observation. This is particularly true in infantile paralysis. The infection in monkeys following inoculation with human material is often detected only after prolonged fatigue by vigorous exercise. The recent report of the production of poliomyelitis in the cotton rat (*Sigmodon hispidus hispidus*) following inoculation with monkey passage material at present seems to be limited to only one strain of human virus. In general, many passages are required for the attainment of fixed virulence and type. With the virus of yellow fever the phenomenon of changing this typical viscerotropic into a fixed neurotropic virus occurs as a result of intracranial inoculations in white mice.

Of added interest is the fact that a given virus, when repeatedly passed serially through a particular species of animal, may increase in virulence for this species and progressively lose its virulence for the original host. The classical example of this change is the rabies virus which when maintained in rabbits becomes highly infectious for these animals but appears to diminish in infectiousness for dogs. Accidental infections in human beings with monkey

strains of the virus of poliomyelitis, as well as rabbit brain rabies vaccine, preclude any generalizations at this time.

Certain findings with other diseases are of considerable interest. Watson, experimenting with the virus of equine encephalomyelitis, succeeded after several failures in establishing the malady in guinea pigs by intracranial inoculations of naturally infected horse brain. Following a few passages it attained high virulence and seemed stabilized as a neurotropic virus for guinea pigs. By similar procedure the guinea pig strain was established and fixed in white mice and, in turn, the mouse strain in the chick embryo. The comparative infectivity and virulence of the three strains so developed in the guinea pig, mouse, and chick embryo, respectively, were significant. When the virus-containing tissues of these animals were titrated in dilutions carried to 1:100,000,000, using 0.1 cc of each dilution as a volumetric dose for inoculation, the minimal infective dose of the guinea pig virus was represented as 1:1,000, the mouse virus as 1:100,000 and the chick virus as 1:100,000,000. That is to say, the guinea pig virus had 50 to 100 times greater concentration and infectivity than the horse virus, the mouse virus 100 times that of the guinea pig virus, and the chick virus 1,000 times that of the mouse virus.

Animals which have recovered from a virus disease or which have survived experimental infection with a virus of modified or attenuated activity exhibit a certain immunity to a subsequent infection with the same virus. The usual antibody responses common to bacteria, such as agglutinins, precipitins, and neutralizing antibodies, are likewise elicited by viruses. In some virus disease the immunity may be of long duration, whereas in other cases the immunity may be relatively short-lived. We have on the one hand smallpox in which immunity usually persists throughout life, and in contrast such virus disease as herpes simplex where frequent recurrence at the site of the preceding lesion is almost the rule. In many instances in which immunity for life seems apparent such as poliomyelitis and measles, the initial resistance may be constantly reinforced by successive exposures; in some virus diseases immunity may be the result of the omnipresence of a definite but limited number of cells infected with the virus. Workers in Dutch Guiana have repeatedly called attention to the solid immunity of the Bush Negroes to malaria and the regularity with which protozoa are distributed in their blood; the so-called "premunition" of the French workers. A similar situation exists in infectious equine anemia of horses, a highly infective but noncontagious disease. The virus is present and apparently multiplies in the circulating blood and has no known special affinity for cellular tissues. The minutest quantity of blood will transmit infection and disease to a healthy horse.

Horses which recover from the disease, and there are many, are apparently immune from further attack but may carry the virus in their blood for the rest of their natural lives and thus become virus reservoirs. As an instance, the blood of a horse which had recovered from the spontaneously appearing disease when about 5 years old, proved highly infective for horses at frequent intervals over a period of fifteen years. Probably one of the most important factors in the epidemiology of virus diseases may be the convalescent carrier.

The artificial stimulation of active earned immunity to virus diseases is dependent seemingly on the development of methods for the production of the virus agents in larger quantities and in a more easily manipulated form. The tissue culture preparations are extremely promising in this direction. On the other hand, passive immunity against virus diseases has made very little progress. The administration to children who have been exposed to measles of convalescent serum or the serum of adults who at one time had measles has imparted protection. But with the neurotropic viruses, such as poliomyelitis, the clinical use of normal adult serum or convalescent serum is of decidedly questionable value, yet *in vitro* such products have exhibited specific neutralizing power. The intracellular position of viruses once they are established, and the lack of penetration by antibodies, are without doubt the mechanism by which the usual beneficial effects of antisera are denied the host.

The portal of entry, the avenue of elimination, and the source of the infection are outstanding unsolved enigmas in connection with many of the virus diseases. Often transmission is by recognized routes, such as the bite of a rabid dog, the bite of an infected mosquito, and the inhalation of infected droplets or spray; unfortunately, in many instances, such information is woefully lacking. One has but to consider poliomyelitis to be impressed with the confusion; there is no consensus of opinion regarding the route by which the virus enters the body of the susceptible host. In addition, an explanation for such a phenomenon as the sporadic appearance of an isolated case of poliomyelitis in a white child in India without seeming contact with any other patient with the disease, which rarely if ever occurs among the natives, worries the epidemiologist.

Perhaps the most intriguing field to those interested in virus research is the possible association of these agents with neoplasms. The evidence seems unequivocal relative to the carcinogenic action of the Shope papilloma virus of rabbits, but the demonstration of a similar etiology in human malignancies is a challenge to the student of virus research.

FUNGI AND FUNGOUS DISEASES*

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THE majority of fungous infections with which the clinician comes in contact are those which produce superficial lesions of the skin. They are caused for the most part by members of the genera *Achorion*, *Microsporum*, *Trichophyton*, and *Epidermophyton*. The widespread infection in this country with these dermatomyces and the consequent familiarity with their clinical manifestations have made the physician aware of their fungous nature. Diagnosis of the etiologic agent can usually be made by direct microscopic examination of scrapings from the lesions. Occasionally this procedure is insufficient for identification. A case in point is the differentiation of *Microsporum audouinii* and *Microsporum lanosum*, both of which produce similar structures in the tissues, but differ culturally. Clinical differentiation is based on the more inflammatory type of lesion produced by *Microsporum lanosum*, but it has been shown by Dowding and Orr¹ that noninflammatory reactions of the tissues with this parasite are not uncommon. In such cases identification of species is necessary before correct therapeutic procedures can be instituted. *Microsporum lanosum* infections respond readily to local treatment with ointments and the like, whereas those due to *Microsporum audouinii* can be treated successfully only after epilation.

Less readily recognized are those mycotic diseases which either occur less frequently or produce systemic infection without an initial local lesion that might give a clue to the nature of the causative organism. A study of the literature reveals the fact that diagnosis in such infections is often delayed considerably, or in the case of a fatal outcome, determined only at autopsy. With the desire to aid the physician and the laboratory worker in the early recognition and identification of the organisms of these obscure mycotic infections, we are presenting a review of the outstanding features of several of them, together with some of our own experimental findings.

CHROMOBLASTOMYCOSIS

The appearance of the lesions in dermatitis verrucosa (chromoblastomycosis) is simulated by several infections caused by different etiologic agents. Clinically it may be confused with tuberculosis cutis, blastomycosis, and at times with Madura foot and coccidioidal granuloma.

The first case of dermatitis verrucosa from which a black fungus, *Phialophora verrucosa*, was isolated was reported by Lane² in 1915. According to Emmons and Carrion,³ Pedrosa in South America had isolated a similar fungus from a case of verrucous dermatitis in 1911, but the case report was not pub-

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lished until 1920. Since these initial reports were made, a number of other cases have been added to the literature, the majority having been found in South America and Puerto Rico where the disease is apparently more common than in this country. Few cases have been reported from the United States. But like other types of fungous infections which are little known in this country, several of which are mentioned in this paper, their rarity may well be due to failure to recognize their fungous nature.

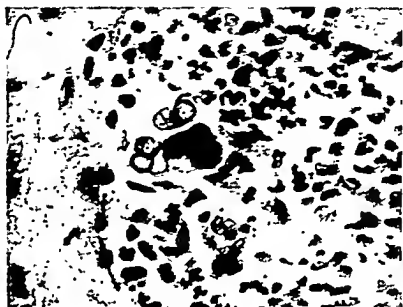


Fig. 1.—*Hormodendrum pedrosoi* in tissue section from a case of chromoblastomycosis of forearm. ($\times 750$.)

The lesions of chromoblastomycosis occur most frequently on exposed surfaces of the body, particularly on the feet. Occasionally other parts of the body may be attacked. Single cases have been reported where the primary infection occurred on the buttocks (Lane²), and on the hand (Carrion,⁴ Martin and associates⁵). Infection may begin as a small, itchy, vesicular skin eruption which gradually extends to produce variously sized areas of infection in adjacent tissues. Wartlike, dull red or violaceous nodules form. These nodules slowly increase in size, forming papillomatous lesions which may become ulcerated. The disease follows a chronic course and does not appear to have the tendency possessed by *Blastomyces dermatitidis* (Gilehrst and Stokes, 1898) to invade other organs of the body.

The term chromoblastomycosis was coined by South American workers to describe the appearance of the organism as seen in tissue preparations. Through common usage this name has gained general acceptance, although the causative agent is not a blastomycete. Differential diagnosis can be made by microscopic examination of scrapings from the lesions which are mounted in sodium hydroxide. The organisms appear as rather large, double-walled, brown, spherical cells. Transverse septa are noted at times. They are distinguished microscopically from *Blastomyces dermatitidis* by their dark color and absence of budding forms and from *Coccidioides immitis* by the absence of endospore formation. Growth takes place slowly when suitable material is transferred to Sabouraud's dextrose agar. Culturally they appear as black colonies with short aerial hyphae, in contrast to the white growth produced initially by *Blastomyces dermatitidis* and *Coccidioides immitis*.

The three species of fungi which have been isolated from the lesions of chromoblastomycosis are *Phialophora verrucosa* (Thaxter, 1915), *Hormodendrum pedrosoi* (Brumpt, 1922), and *Hormodendrum compactum* (Carrion, 1935). Differentiation has been based on the size, shape, and mode of production of the spores in cultures. In the tissues, however, the cells of all three species have a similar appearance. Clinically the lesions produced by *Hormodendrum compactum* differ from those usually seen in chromoblastomycosis only in the absence of the nodule formation.⁴ This species distinction is of interest mainly to the mycologist.

The disease, once having become extensive, is generally considered incurable, but the slowly progressive nature of the infection should allow opportunity for experimental work in the therapeutic field. Moderately successful results have been obtained by Carrion and Koppisch⁶ who noted extensive healed areas and a reduction in the size of the nodules after intravenous treatment with large doses of sodium iodide. Martin and co-workers⁵ reported general clinical improvement of the skin lesions following daily iontophoresis treatment with copper sulfate for a period of five months. A marked reduction in the number of fungi in the lesions occurred. Oral administration of potassium iodide and autogenous vaccine therapy, which was also tried in this case, was considered of questionable value due to the small amounts given.

TORULA MENINGITIS

Central nervous system infection with a yeastlike organism, *Torula histolytica* (Stoddard and Cutler, 1916) was first reported by von Hansemann⁷ in 1905 from Germany. Since that time, additional cases with a wide geographic distribution have been observed, bringing to a total of sixty-four the number of reports now published. Particularly excellent reviews of many of these cases have been written by Freeman⁸ and Levin.⁹

The protean clinical manifestations of meningoencephalitis due to *Torula histolytica* have made the diagnosis of this disease a difficult one. This, and the apparent failure of the clinician to bear in mind the possibility of the invasion of the central nervous system with this organism, have frequently been responsible for such initial diagnoses as brain tumor, tuberculous meningitis, encephalitis, or cerebrospinal syphilis. Because of this, several writers have raised the question as to whether this infection may not be less rare than the number of cases would lead one to believe.

Obviously, in a disease which offers such a variety of clinical impressions, a definite picture cannot be given, but certain symptoms have been commonly reported. At the onset there is an intermittent, frontal headache, often increasing in severity. Weakness, dizziness, nausea, vomiting, and disturbances of vision are frequently noted. The patient is usually afebrile, or at most, a low-grade fever is present. Gradual loss of weight and strength are manifest during the course of the disease, the duration of which may vary from several weeks to a number of years before death occurs.

Ante-mortem diagnosis can be made only by examination of the spinal fluid. The cell count usually varies between 100 and 500, with a predominance of lymphocytes. Yeast cells may be seen at times in the unstained preparations, but more satisfactory examination of the spinal fluid can be made with Gram's

stained or India ink preparations. In the former, the yeast cells appear as gram-positive oval cells from 5 to 8 microns in diameter; budding forms may be seen occasionally. The India ink preparation offers the advantage of making the capsular material plainly visible. This capsule appears as a clear halo surrounding the thick cell wall and distinguishes the cells of *Torula histolytica* from those of the *Monilias* with which they might be confused. But the presence of a capsule is not alone sufficient proof of their pathologic significance. Further study of the organism should be made by culture and animal inoculation.

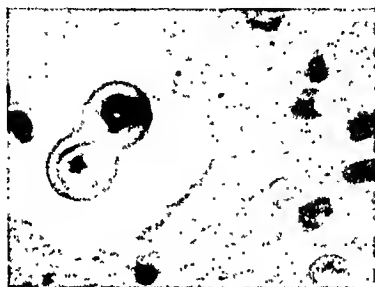


Fig. 2.—Budding cell of *Torula histolytica* in tissue section of mouse brain. ($\times 3200$.)

The centrifuged spinal fluid sediment should be streaked on Sabouraud's dextrose agar plates and incubated at either 37°C . or at room temperature. The initial growth occurs in from forty-eight to seventy-two hours as small, smooth, moist, cream-colored colonies which become tan or brown upon aging. The cells in the culture are variable in size, ranging from 2 to 10 microns in diameter, although larger cells may be seen. Budding cells are numerous. The capsules can be demonstrated in the cultural material by the India ink method. Large capsules are found most frequently in the more virulent strains.¹⁰ Sugars are usually not at all, or only feebly, fermented.

Mice and rats are the most susceptible of the laboratory animals to infection with *Torula histolytica*. Rabbits and guinea pigs are less so. Intraperitoneal inoculation of a heavy saline suspension of the culture into mice results in a generalized infection with constant involvement of the brain and meninges.¹¹ Death of the animal usually occurs in from six to eight days, but life may be prolonged for several weeks or months.¹² At autopsy encapsulated forms can best be demonstrated from the abdominal fluid and less readily so from the lungs, liver, spleen, and brain, although the organisms are numerous in these organs.

The route by which *Torula histolytica* first gains entrance into the human body is not known. The general opinion is that the respiratory system is the most likely point of initial invasion.

As yet there is no satisfactory treatment for the infection when it occurs in the central nervous system. Intravenous and oral administration of iodides has been tried without beneficial effect. All cases to date have been fatal.

SYSTEMIC HISTOPLASMOSIS

The first three cases of a new disease caused by the parasite, *Histoplasma capsulatum*, were reported by Darling¹³ from Panama between the years 1906 and 1908. Due to the appearance of the organism in the tissues obtained at autopsy, the disease was believed to be a protozoan one bearing a resemblance to kala-azar. Not until DeMonbreun¹⁴ in 1934 cultivated the parasite from autopsy material was the fungous nature of the disease discovered. Since Darling's initial reports, ten additional cases, all occurring in this country, have been added to the literature. Nine of these cases have been reported within the last six years, which may indicate that familiarity with the clinical symptoms has resulted in the recognition of cases that may have been missed previously.

The disease affects the reticulo-endothelial system, and it has been suggested by DeMonbreun¹⁴ that cytomyeosis of Darling rather than histoplasmosis of Darling would best describe the nature of the infection. However, the name histoplasmosis has priority and most cases have been reported under this terminology.

The onset is of an insidious nature, the chief complaint being weakness and loss of weight. Severe sweats, particularly at night, may occur. Splenomegaly, hepatomegaly, irregular fever, secondary anemia, leucopenia, and enlargement of the superficial lymph nodes are characteristic findings. In cases with the above-mentioned symptomatology, the possibility of infection with *Histoplasma capsulatum* should be considered.

Diagnosis can be made by finding the parasite within the cytoplasm of the large endothelial-type cells in blood smears or sternal bone marrow smears. When stained by Wright's method, they appear as bluish or bluish-red, oval cells, from 2 to 4 microns in diameter, surrounded by a clear capsular-like substance. The cytoplasm is characteristically situated along the side walls or at one end of the cells, but the absence of any body resembling a blepharoplast distinguishes this parasite from the Leishman-Donovan bodies found in kala-azar, with which it might be confused. Few or many of the parasites may be found within a single cell. Near the terminus of the infection they are phagocytosed by the polymorphonuclear cells also. The stage of the disease at which the parasites appear in the blood in sufficient number to be readily found is not known, since in only two cases (Dodd and Tompkins,¹⁵ and Reid and co-workers¹⁶) has antemortem diagnosis been made by blood smear examination. In each case the diagnosis was made late in the infection.

The organisms can be cultivated on such media as Sabouraud's dextrose agar, blood agar slants, or semisolid veal infusion blood agar. Possibly earlier diagnosis might be made by the culture of the blood because of the larger amounts that can be examined. We have had some indications that this may be true from animal experiments that have not yet been published. Two growth phases of the organism occur, depending upon the media used and the temperature at which the cultures are incubated. In semisolid media or on blood agar incubated at 37° C., a yeastlike form develops within from six to ten days and may be confused at first with other species of fungi, such as the *Saccharomyces* or *Torula*, as mycelia do not form readily under these growth conditions. A mycelial type of growth occurs when transplants of the yeast form are made

to Sabouraud's dextrose agar and incubated at room temperature. Small colonies, with aerial hyphae which give them a downy appearance, are visible, usually within a week. The gross appearance of the colony is not diagnostic and portions of it should be examined in wet mounts for the developing arthrospores. The older spores of *Histoplasma* and related species have rather typical tubercle-like processes which extend out on all sides of the cell wall giving them the appearance of "ancient Teutonic warelubs."¹⁴

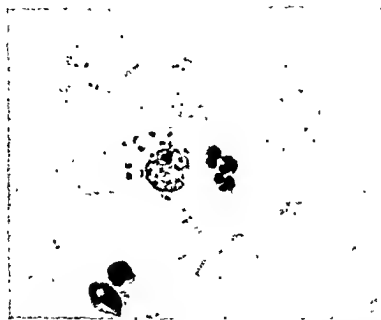


Fig. 3.—Sternal bone marrow smear from a case of histoplasmosis, showing a reticuloendothelial cell containing numerous parasites within its cytoplasm. (Wright's stain, $\times 1000$.)

Determination of the pathogenic nature of the parasite can be made by injection of the yeastlike form into suitable experimental animals. Intraperitoneal inoculation into guinea pigs produces a chronic, afebrile, systemic disease with pathologic changes not unlike those found in man.¹⁵ The animals can be autopsied after the second week of infection. Stained smears or tissue sections of the spleen, liver, lungs, and bone marrow should be examined for the parasites which are similar in appearance to those found in the tissues of infected human beings. The fungus can also be cultivated from the tissues. Monkeys are also susceptible to infection, and puppies and mice less so.¹⁴ Rabbits have thus far proved refractory to infection.

As only two cases have been diagnosed during life, there is little that can be said as to the effectiveness of specific therapy. Symptomatic treatment with sodium bicarbonate and blood transfusions as reported by Dodd and Tompkins¹⁵ gave only temporary improvement. In the case recorded by Reid and his associates,¹⁶ oral administration of potassium iodide proved of no value. As the diagnosis in both of these cases was made after the parasite had invaded the body in massive numbers, little could be expected from the therapeutic measures that were tried. In a case reported by Clemens and Barnes,¹⁷ in which diagnosis was made at autopsy, sulfanilamide was given a brief trial, but the absence of clinical improvement caused them to discontinue this form of treatment. That the use of arsenicals might have a beneficial effect has been suggested by Shaffer, Shaul, and Mitchell.¹⁸ Their opinion is based on the results obtained with arsphenamine by Nègre and Bidre in the therapy of human epizootic lymphangitis. "Since some writers have maintained that there is

similarity between *Cryptococcus farciminosus*, the etiologic organism of epizootic lymphangitis, and the parasitic agent of histoplasmosis, one of the arsenicals might theoretically be indicated in treatment of the latter."

SUMMARY

The clinical symptoms and laboratory procedures of use in the diagnosis of several of the less commonly recognized mycotic infections, chromoblastomycosis, torula meningitis, and systemic histoplasmosis, are presented.

REFERENCES

1. Dowding, E. S., and Orr, H.: The Dermatophyte *Microsporum Lanosum*, *Mycologia* 51: 76, 1939.
2. Lane, C. G.: A Cutaneous Lesion Caused by a New Fungus (*Phialophora verrucosa*), *J. Cutan. Dis.* 33: 840, 1915.
3. Emmons, C. W., and Carrion, A. L.: *Hormodendrum Pedrosoi*. An Etiological Agent in Chromoblastomycosis, Puerto Rico *J. Pub. Health & Trop. Med.* 11: 639, 1936.
4. Carrion, A. L.: Chromoblastomycosis. A New Clinical Type Caused by *Hormodendrum Compactum*, Puerto Rico *J. Pub. Health & Trop. Med.* 11: 663, 1936.
5. Martin, D. S., Baker, R. D., and Conant, N. F.: A Case of Verrucous Dermatitis Caused by *Hormodendrum Pedrosoi* (Chromoblastomycosis) in North Carolina, *Am. J. Trop. Med.* 16: 593, 1936.
6. Carrion, A. L., and Koppisch, E.: Observations on Dermatomycosis in Puerto Rico, Puerto Rico *J. Pub. Health & Trop. Med.* 9: 169, 1934.
7. von Hansemann: Über eine bisher nicht beobachtete Gehirnerkrankung durch Hefen, *Verhandl. d. deutsch. path. Gesellsch.* 9: 21, 1905.
8. Freeman, W.: Torula Infection of the Central Nervous System, *J. f. Psychol. u. Neurol.* 43: 236, 1931.
9. Levin, E. A.: Torula Infection of the Central Nervous System, *Arch. Int. Med.* 59: 667, 1937.
10. Benham, R. W.: Cryptococci—Their Identification by Morphology and by Serology, *J. Infect. Dis.* 57: 255, 1935.
11. Crone, J. T., DeGroat, A. F., and Wahlin, J. G.: Torula Infection, *Am. J. Path.* 13: 863, 1937.
12. Holt, R. A.: Personal communication.
13. Darling, S. T.: Histoplasmosis; A Fatal Infectious Disease Resembling Kala-Azar Found Among Natives of Tropical America, *Arch. Int. Med.* 2: 107, 1908.
14. DeMonbreun, W. A.: The Cultivation and Cultural Characteristics of Darling's Histoplasma Capsulatum, *Am. J. Trop. Med.* 14: 93, 1934.
15. Dodd, K., and Tompkins, E. H.: A Case of Histoplasmosis of Darling in an Infant, *Am. J. Trop. Med.* 14: 127, 1934.
16. Reid, J. D., Scherer, J. H., Herbut, P. A., and Irving, H.: Accepted for publication.
17. Clemens, H. H., and Barnes, M. L.: Histoplasmosis of Darling, *South. M. J.* 33: 11, 1940.
18. Shaffer, F. J., Shaul, J. F., and Mitchell, R. H.: Histoplasmosis of Darling, *J. A. M. A.* 113: 484, 1939.

MEDICAL PROBLEMS OF HIGH ALTITUDE FLYING*

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HISTORICAL DEVELOPMENTS

IT IS interesting to note that while airplanes are of relatively recent origin, medical studies of high altitude flying are not. During one of the earliest balloon flights, on December 17, 1783, to be exact, the balloonist reported severe pains in his ears—the first of a long series of complaints which have during the intervening 157 years gradually led to the establishment of aviation medicine as a recognized specialty of medical science.

In 1862 Glaisher and Coxwell made a balloon ascent to an altitude of approximately 29,000 feet, during which the former noticed a series of strange symptoms marked by loss of visual acuity and hearing, paralysis of the legs and arms, and finally by unconsciousness. At the same time his companion Coxwell also found that his arms were paralyzed but had the presence of mind to seize the valve rope with his teeth and start the balloon downward. Glaisher's published accounts of this and other flights came to the attention of Paul Bert, a brilliant French physiologist, who began a study of the effect of decreased barometric pressures.

Three years later Bert¹ published his famous "*La pression barometrique.*" This work dealt with increased as well as with decreased atmospheric pressures, and the number and accuracy of his experiments and deductions considering the facilities with which he had to work are astounding. Bert was the first to prove that the principal effect of high altitude was due to the decreased partial pressure of oxygen; he carried out innumerable researches concerning carbon dioxide in the lungs and blood, and probed deeply into the question of respiration and blood gases under decreased barometric pressures. In addition, he observed many mechanical effects of barometric pressure changes.

Following the work of Bert there was little or no further work done in this field until about the last year of World War I. At the outbreak of that war the airplane was not well developed, and it was 1916 before its value as a war weapon was fully utilized. With the improved performance of the war planes came the problem of oxygen want at high altitude. While there were many in the different countries who studied this problem, their investigations were all overshadowed by the researches of E. C. Schneider, who worked in the Army Air Service, Medical Research Laboratory at Mineola, Long Island, from 1918 to 1920.

RECENT DEVELOPMENTS

Between 1920 and 1935 there were but few medical studies on high altitude flying. Since 1935, however, the amount accomplished has been tremendous.

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The stimulus that brought about this renewed medical research arose from several sources. The principal one of these was the marked climbing ability of our newer type airplanes and the desirability of operating at higher levels, which has certain marked advantages in both civil and military aviation. In civil aviation airplanes develop more speed on less power at altitude, and at the higher levels much of the stormy weather and bumpy air is avoided, contributing both to the comfort and to the safety of the flight. In military aviation the same considerations hold true. In addition altitude is an excellent means of concealment and protection from enemy aircraft and antiaircraft artillery fire.

The rate of climb and the altitude to which a commercial air liner may go are limited, however, not by the performance of the airplane but by federal regulations designed to protect the passenger. At the present time the allowable maximum altitude is 18,000 feet. Thus great care must be exercised during the discussion to follow to distinguish between the effects of high altitude flying on commercial passengers, limited by regulations as indicated above, and the effects on military pilots, limited only by the performance of the airplane or the tolerance of the human organism.

A second factor important in this connection is the number of persons who may be involved. As of January 1, 1940, there were 31,000 licensed civil pilots in this country, and about 20,000 more in training. By January 1, 1941, it is estimated that the number of licensed civil pilots will be increased to 50,000 and by July 1, 1943, to 100,000. In military aviation the number of pilots and combat crew members is approximately 10,000, which in the event of a national emergency would quickly be increased to well over 100,000. By far the largest number of fliers is composed of those commercial passengers carried by air. In the past twelve-month period this number amounted to approximately 3,500,000 persons, and represents an increase of 43 per cent over that of the year previous. At this rate of increase we may expect over 20,000,000 persons to be transported in scheduled and unscheduled air transport operations during the next five years.

CURRENT PROBLEMS

The current medical problems of high altitude flying depend principally on the altitude attained. As a consequence, the problems of commercial air transport operations are fewer and less serious than those in military aviation, since the former is limited to 18,000 feet. In either instance all the deleterious effects of altitude flying are brought about by the decrease in atmospheric temperature and pressure with ascent.

The temperature of the atmosphere decreases about 2° C. for each 1,000 feet of ascent until 35,000 feet has been reached. Above that altitude the temperature remains constant at -55° C.

In spite of winter-flying clothing, personnel begin to suffer at about -10° C. The hands and feet are the first parts affected, followed by the back, chest, abdomen, and legs in order. At first there are chilly sensations accompanied by increased metabolism and muscular restlessness. These chilly sensations change to discomfort, and the acuity of touch sensations and muscular reactions is dulled. As the cold increases, there is numbing of the parts, producing clumsiness of movements. The muscles assume a state of mild tonic contraction.

which further hampers and restricts free movement. Discomfort changes to pain and generalized shivering appears. Voluntary muscular movements become sluggish, and finally tissue destruction and death may occur at temperatures of -25° to -40° C.

Very recently Grow² has added a considerable amount of data to our knowledge of the effect of cold. His attention was first attracted to this problem when he observed that the mental state of persons suffering from exposure to cold was similar to that of those suffering from anoxia. By animal and human experiments he was able to show that such exposure produced lowering of the body temperature, slowing of the circulation and respiration, lowering of the oxygen content of the arterial blood (of animals), and tissue anoxemia of both animals and man. These findings are particularly significant in aviation due to the fact that anoxemia may also occur from decreased atmospheric pressure at high altitudes.

The solution to the problem of cold in high altitude flight is to close and heat the cabin. This has been satisfactorily accomplished in civil aircraft and to a certain extent in military aviation.

ANOXIA

During ascent the composition of the atmospheric air remains constant but its total pressure and partial pressure of its component gases decrease. This decrease in turn brings about a decrease in the alveolar oxygen tension and in the oxygen saturation of the arterial blood. This results in a tissue anoxia to which, in aviation, the term "altitude sickness" has been given.

Objective Symptoms.—During ascent to high altitude there is a point at which breathing becomes altered. This varies in different individuals, but it has been noted as low as 4,000 feet. At about 12,000 feet altitude the increased depth in breathing amounts to between 20 and 100 per cent increase in lung ventilation. With further ascent there is a greater increase in depth of breathing, and at around 20,000 feet each inspiration may amount to 600 to 1,200 c.c. of air. The rate of respiration changes very little at any altitude and even under extreme conditions seldom increases more than 5 breaths per minute.

Death from altitude sickness in the normal organism is probably always due to a failure of the respiratory center. This may occur at different altitudes, depending on the duration of exposure. It is believed that an ascent to about 16,000 feet might eventually prove fatal in some cases. Above this altitude the exposure period necessary to produce death is progressively shortened, such that at 25,000 feet altitude and above, death may occur at any time after twenty to thirty minutes.

Most of our knowledge concerning the response of the cardiovascular system to altitude has been worked out by Schneider and Schneider and Lutz.³ They found that there were two types of reactors: "fainters" and "nonfainters." Of those tested 46.7 per cent were in the former classification and 53.3 per cent in the latter.

The fainters are those in whom the lower centers, which control heart rate, vascular tone, and the rate and volume of breathing, suffer paralysis before the higher or psychic centers are affected.

The nonfainters exhibit a failure of the psychic centers before the cardiac, vasomotor, and respiratory centers become seriously affected. A majority of these individuals sit erect and the physiologic processes continue to function normally for a few moments after consciousness is lost.

During ascent the pulse first begins to react at widely different intervals between 4,000 and 14,000 feet altitude. From the initial point of reaction on, the pulse tends to increase until the limit of endurance is reached when the mean increase of nonfainters is 28 beats, and that of fainters is 26 beats per minute. Accelerations of 62 beats per minute have been noted.

The blood pressure, like the pulse and respiration, may show a beginning change at 4,000 feet altitude. During ascent nonfainters may show a gradual rise of systolic pressure at altitudes of between 8,000 and 12,000 feet, but beyond that level there is a slow fall with increase in pulse pressure. In fainters the blood pressure is characterized by a sudden terminal diastolic fall. At a sustained altitude of 8,000 to 16,000 feet the blood pressure tends to return to normal values.

The effect of altitude on the heart is not, as many persons believe, due principally to the increased load but almost entirely to the lack of oxygen supply to the heart muscle. While it is true that the pulse rate increases and that in some cases the blood pressure rises, these changes are no more noticeable than would be seen in mild exercise. The effect of anoxia on the heart muscle is the same as that for any other tissue and, as pointed out above, the heart will continue to function long after the respiratory centers have become paralyzed. The contention that dilatation of the heart frequently occurs from moderate degrees of anoxia has been found in error.

The electrocardiographic changes from anoxia consist essentially of a lowering or inversion of the T-waves, a depression of the R-T interval, and sometimes a deformity of the QRS group.

With reference to the problem of flight with an abnormal heart we know from the experimental results obtained by Schneider and Lutz,³ Graybiel, and others,⁴ that anoxia has a detrimental effect. The seriousness of the effect is proportional not only to the altitude and the duration of exposure but also to the amount of cardiac impairment. The type of heart lesion does not seem to be as important as the amount of cardiac reserve, nor does the increased demand on the heart seem to be as important as the effect of anoxia on the heart muscle.

The power of the external ocular muscles has been found to be decreased above 15,000 feet. Above 15,000 feet visual acuity and light perception become lessened, but some vision is retained up to the point of unconsciousness.

Hearing is usually not affected as quickly as vision, and 16,000 to 18,000 feet may be reached before this sense is noticeably affected. Above this level, however, it decreases rapidly, and at 20,000 feet the noise of the airplane motor may be entirely imperceptible.

At altitudes of about 14,000 feet and above there is a progressive deterioration of voluntary muscular control. The first sign to appear is incoordination of the finer muscular movements, followed by slowing of movements, tremor, and finally paralysis.

By far the most interesting and the most striking objective symptoms of altitude sickness are those due to a change in behavior. These have been studied extensively by McFarland⁵ both in flight and in the laboratory.

Most recent writers agree that there are no significant psychologic changes from anoxia even during prolonged flights at altitudes below 9,000 feet. Above this altitude, however, psychologic changes begin to appear, and the degree of change, as in the case of physiologic processes, is in proportion to both the altitude and the duration of exposure. These effects consist of a slight decrease in immediate memory and the onset of psychologic complaints. At about 14,000 feet altitude handwriting begins to become impaired, and there may be a change in moods, more or less characteristic in each individual; there may be sleepiness, lack of volition, and lethargy, or there may be a temporary stimulation with euphoria.

At about 18,000 feet the effects become much more pronounced. At this point, in addition to a definite decrease of sensory perception, the impairment of neuromuscular control is quite marked, the field of attention is narrowed, and the higher mental processes are affected, as indicated by the loss of memory, the development of irrational or fixed ideas, and the loss of sound judgment and self-criticism.

Between 20,000 feet and the point at which unconsciousness occurs, the psychologic effects are very profound. Simple reaction time tests and simple sensory and motor responses become altered. There is a marked loss of neuromuscular control, and there may be twitching, rhythmical movements, or even paralysis. Awareness of the lapse of time is lost, as well as volitional control, except that there is a great persistency in attempting to carry out an assigned task. Explosive emotional outbursts may occur, with periods of laughter, anger, or pugnaciousness.

Subjective Symptoms.—There are few, if any, other conditions known to medicine which produce such profound changes in the body, or which may even produce death without creating more pronounced subjective manifestations than those experienced in acute altitude sickness. In fact, it is not at all unusual for a person to become unconscious from the effects of the latter without being aware of any change from the normal. However, some individuals do experience various mild subjective symptoms during an attack of altitude sickness, and many suffer more or less afterwards.

At altitudes of 12,000 feet or above individuals may react psychologically in one of two general ways. Some feel tired, depressed, or sleepy, and tend to fall asleep. Others, however, will have the opposite reaction and become euphoric, with outbursts of hilarity, uncontrolled laughter, or pugnaciousness which do not appear ludicrous or unnatural to the individuals concerned, although they are usually aware of their moods. At an altitude of about 25,000 feet coma occurs. This comes on rather suddenly but not unpleasantly and usually without any subjective warning.

In contradistinction to the relatively mild or even total absence of subjective symptoms while at high altitude, the after effects may be very severe. This appears to be due to the fact that the sensory functions of the body return to

normal almost immediately upon returning to low altitude, while the other general systemic effects tend to persist for some time afterwards, provided the anoxia has been very severe, or even in mild cases if it has been prolonged.

Flying at 10,000 to 12,000 feet for a period of two to four hours is usually followed by a dull headache and a sense of fatigue both of which may persist for several hours, the fatigue generally being the more prolonged. As the length of the flights at the above altitudes increases, the aftereffects increase in proportion both in intensity and duration.

Exposure to an altitude of 15,000 to 18,000 feet for a period of two to six hours may be followed by a very severe intractable headache, nausea, vomiting, dizziness, mental confusion, muscular weakness, and even complete prostration. At higher altitudes these same effects may appear with shorter exposures, until at about 24,000 feet fifteen or twenty minutes may be all that is required to produce them. The more severe the symptoms the longer they persist, and as long as forty-eight to seventy-two hours may be required for complete recovery.

If oxygen instead of air is breathed at high altitude, the partial pressure of the oxygen can be maintained normal and altitude sickness prevented up to 33,000 feet. Above that altitude the partial pressure drops below normal and reaches zero at 50,300 feet,⁶ even while breathing pure oxygen. Between these two altitudes all the symptoms of altitude sickness occur, as described above, with unconsciousness appearing at about 43,000 feet. From this it is evident that even with oxygen high altitude flying is definitely limited.

DECREASED ATMOSPHERIC PRESSURE

The decrease of atmospheric pressure with ascent may produce five different pathologic states in the body. Three of these are brought about by expansion of the gases contained in the body cavities.

Probably the most frequent difficulty experienced by passengers and pilots in flight is with reference to the middle ear. The middle ear is an air-filled cavity which is ventilated through the eustachian tube. The eustachian tube is normally collapsed with its walls in apposition under ordinary circumstances and is open only during swallowing, yawning, and other physiologic acts. As a consequence, during a change of altitude either a positive or a negative pressure builds up in the middle ear unless the eustachian tube is voluntarily opened. If for any reason the eustachian tube is stenosed, which may occur even from a mild coryza or pharyngitis, the middle ear cannot be ventilated and a marked change of altitude may then rupture the eardrum.

A like condition occurs with reference to the sinuses when their openings are blocked, in which case, during a change of altitude, marked trauma and severe pain may develop.

Another condition which may occur is abdominal distention, since the gastrointestinal gases expand in direct proportion to the decrease of atmospheric pressure during ascent.

Since the above-mentioned effects may occur in normal individuals, it is obvious that those suffering from various pathologic states may be even more adversely affected. Thus any inflammation or infection of the middle ear or the

nasal accessory sinuses may be aggravated by flight. Expansion of the gastrointestinal gases will, of course, affect those with hernia, recent abdominal operative wounds, gastric or duodenal ulcer, acute or chronic appendicitis, or obstruction of the intestinal tract.

The fourth pathologic state which may arise in high altitude flight is *aero-embolism*,⁶ a clinical condition in aviation corresponding to compressed air illness in deep sea diving. That the compression of an individual followed by a rapid decompression would result in illness has been recognized for over one hundred years. The exact cause of this condition was not definitely known, however, until the investigations of Bert were published in 1878. Bert showed that the symptoms were due to the liberation of a gas in the body and that this gas was composed chiefly of nitrogen. *Aero-embolism* is a condition caused by the same general process that causes compressed air illness, with the exception that the former occurs from compression to 2 or more atmospheres followed by decompression, while the latter occurs from decompression from 1 atmosphere pressure or less.

During ascent in aircraft, or in any other situation in which the atmospheric pressure is decreased, the internal partial pressure of the body nitrogen is above that of the nitrogen in the lungs, and the tissues are, therefore, supersaturated. As a consequence, the nitrogen dissolved in the blood begins to be liberated in the lungs, the nitrogen in the tissues begins to enter the blood stream, and by this dual process the body tends to rid itself of its excess nitrogen. If the ascent is slow enough so that the nitrogen in the body can be eliminated and not reach approximately double its normal saturation at the prevailing altitude, nothing unusual will occur. If, on the other hand, concentration of nitrogen in the body becomes more than double its normal saturation value at any altitude pressure, the nitrogen gas will come out of solution and form bubbles to which is added some oxygen, carbon dioxide, and water vapor from the surrounding blood and tissues.

Since the elimination of nitrogen from the body is entirely through the blood stream, those parts of the body or those tissues which have the poorest blood supply will be the least able to lose their excess nitrogen. The blood is the first to lose its excess nitrogen, the body tissues (other than fatty tissues) second, and the fatty tissues last, the delay in the latter being due to both its higher nitrogen content and to its poorer blood supply. That this is essentially correct is indicated by the fact that with rapidly decreased atmospheric pressures, gas bubbles are found in the spinal fluid and about the spinal cord at 18,000 feet altitude, while bubbles in the blood and body tissues generally have not been found below 30,000 feet altitude.

It is obvious that if one ascended slowly enough to high altitude, the nitrogen of the body would be eliminated as fast as it tended to become excessive. Therefore, the rate of ascent is important, since the nitrogen content of the tissues must be at least twice normal at any given atmospheric pressure before it will appear as bubbles. During ascent bubbles first appear in the spinal fluid, but at the present time no experimental data have been obtained which give the maximum rate of ascent that can be used without these bubbles being

formed; it has been calculated that they would develop at any ascent of more than 78 feet per minute.

The symptoms of aero-embolism are identical with those of compressed air illness and consist principally of joint pains, paralysis, paresis, hyperesthesia, embolism, pulmonary edema, and formication, neurodermatitis, or pruritus of the skin.

The treatment, of course, is the returning of the patient to a region of higher atmospheric pressure, which in the case of high altitude flight simply involves a return to lower altitudes.

In addition to active treatment there is a prophylactic measure available which may be utilized. This consists of the inhalation of pure oxygen for one-half to one hour before ascent. This eliminates most of the nitrogen from the body and hence the cause of the disease.

FUTURE TRENDS

That the cold of high altitude can be controlled by closing and heating cabins, that altitude sickness can be controlled up to 33,000 feet by the breathing of oxygen, and that attacks of aero-embolism can also be controlled by the breathing of oxygen have been indicated. A new method of controlling these problems, together with all other clinical entities peculiar to high altitude flight, has recently reached the stage of practical development. This involves the use of a pressure cabin. It will be recalled that, except for cold, all medical problems of high altitude flight arise either directly or indirectly from a decrease of atmospheric pressure. From this it should be evident that if an airplane cabin were sealed pressure-tight and the contained air maintained at or near sea level pressure, all these problems would cease to exist. This theory has been tested and found to be successful.

While pressure-cabin aircraft may differ greatly in details of construction and operation, they all incorporate the following general features: Openings in the cabin walls, such as windows, doors, and control cables, etc., are sealed airtight against the internal positive pressure. An air compressor of some type is used to take in atmospheric air, compress it, and force it into the cabin under pressure. A cabin exhaust valve is provided to transmit the air in the cabin back to the outside atmosphere and thus allow the entrance of fresh air from the compressor. Strangely enough this system also takes care, to a large extent at least, of the high altitude cold, for the incoming air is heated by compression.

The pressure cabin is ideally suited for use on commercial air lines from the passenger standpoint, since it not only eliminates the oxygen question but also does away with distressing symptoms from the ears and other parts of the body due to barometric pressure changes. From the operator's standpoint greater speed and economy can be obtained by high altitude flight, provided the flights are of sufficient length. Based on present trends, it is believed that transcontinental and transoceanic aircraft will practically all be of the pressure-cabin type within the next five to eight years, and that within ten to twelve years practically all transport lines will operate pressure cabins exclusively. This latter is based on the fact that the ear and sinus problems in passengers will

always be with us at high or low altitudes until the pressure of the cabin is controlled within a very narrow range. The pressure-cabin airplane is easily the greatest contribution to human comfort and safety in flight that has ever been developed.

REFERENCES

1. Bert, P.: *La pression barometrique*, Paris, 1878, Masson et Cie.
2. Grow, M.: Preliminary Report of Effect of Cold on Oxygen Content of the Blood, *Mil. Surgeon* 86: 225, 1940.
3. Schneider, E. C., and Lutz, B. R.: Circulatory Responses to Low Oxygen Tension, *Air Service Medical*, Washington, D. C., Govt. Printing Office 1: 86-98, 1920.
4. Graybiel, A., Missiuro, V., Dill, D., and Edwards, H.: Experimentally Induced Asphyxiation in Cardiac Patients With Special Reference to Certain Hazards in Air Travel and to Use of Asphyxiation as Cardiac Function Test, *J. Aviation Med.* 8: 178, 1937.
5. McFarland, R.: The Effects of Oxygen Deprivation on the Human Organism, Department of Commerce Report No. 13, 1938.
6. Armstrong, H. G.: *The Principles and Practice of Aviation Medicine*, Baltimore, 1939, Williams & Wilkins Co.

METHODS OF RESUSCITATION*

RALPH M. WATERS, M.D., MADISON, WIS.

IF IT exists, the opportunity to bring the dead to life is rare. The sudden acute failure of the function of respiration or of circulation, or both, may result in a condition resembling death from which recovery is possible if proper measures are promptly instituted. It is to such "proper measures" for the restoration of respiration or circulation that the term "resuscitation" is, rightly or wrongly, often applied. The abundant margin of safety maintained by nature in the case of almost every other physiologic function is lacking in the matter of the transport of oxygen from environmental atmosphere to the tissues. Continuous adequate breathing, together with adequate blood flow, constitutes this transport mechanism. The total failure of either respiration or circulation must be extremely brief if the individual is to survive.

The length of time during which interruption of oxygen transport may be compatible with complete recovery has been the subject of much speculation. It depends upon the condition of the cells at the instant of complete interruption of transport and varies from a few seconds to nine or ten minutes. The individual suffering from severe cardiac decompensation may die within fifteen seconds of the initiation of complete respiratory obstruction or after three breaths of pure nitrous oxide. At the other extreme, a healthy athlete, after a period during which he breathes pure oxygen, may safely stay under water for nine or ten minutes. Recovery of the vegetative functions has been reported following periods of arrested oxygen transport of considerable duration.^{1, 2} The more specialized centers of the central nervous system tolerate oxygen deprivation poorly. Failure of recovery may be preferable to recovery accompanied by blindness, amnesia, idiocy, or other defects of the higher centers. Methods of resuscitation to be effective must be instituted promptly and must transport oxygen efficiently until normal activity is restored.

Fisher³ traced the evolution of methods of resuscitation. He described in more or less detail, almost a hundred methods, 77 of which were attempts to perform artificial respiration, and 18 were designed to re-establish respiration by sensory stimulation of one sort or another. With the exception of cardiac or vascular massage, which is performed purposely or incidentally in many methods of artificial respiration, Fisher mentioned only four procedures involving direct attack upon the circulatory system.

Other than by the replacement of deficient blood volume by intravascular fluid injection, it is probable that less than 4 per cent of candidates for resuscitation can be benefited by direct attempts to replace, restore, or stimulate the cir-

*From the Department of Anesthesia, University of Wisconsin Medical School.

culatory mechanism. The surgical nature of direct attack upon the heart or blood vessels makes for delay and may interfere with efficient artificial respiration. The intravascular, intracardiac, or other administration of so-called analeptics, when the tissue cells already suffer oxygen want, may add insult to injury. The application of the electric current to re-establish heart action, suggested by Snow⁴ and others⁵; the desensitization of the fibrillating heart by topical application or vascular injection of procaine, as suggested by Beck⁶; the intracardiac injection of adrenalin, and other specialized procedures demand knowledge and equipment not likely to be available under the usual circumstances when methods of resuscitation are needed.

Prompt restoration of oxygen, then, to the cells of the central nervous system is our primary object. Except in very unusual circumstances, the introduction of oxygen into the circulation other than through the alveolocapillary membrane^{7, 8} has not proved satisfactory. Prompt, efficient, and yet not traumatic or overvigorous, artificial respiration is, therefore, the sheet anchor of resuscitation.

ARTIFICIAL RESPIRATION

If there are 77 different methods of performing a technical procedure (and Fisher did not exhaust the literature by any means¹), it indicates one of two things. Either no known method is satisfactory, or the method is of little importance compared with the understanding with which the method is applied. The latter may be presumed to be the case with methods of artificial respiration. Success was reported by the originator of each of the 77 methods. Why? Undoubtedly some of the individuals "resuscitated" would have re-established adequate oxygen transport without treatment. However, the originator of a method in every case had devoted time and thought to the problem involved. His effort to maintain a substitute for normal respiration was performed intelligently, and the result was satisfactory. However vigorously the chest be "squeezed" or the upper air passages "blown into"—be it by hand or machine—unless oxygen is replenished in the alveoli, the result will be valueless. Not infrequently has an efficient method of artificial respiration been applied in the presence of an obstructed glottis and with negative results. All too frequently do we waste valuable seconds or even minutes in procuring a piece of mechanical apparatus or a cylinder of oxygen to treat respiratory failure, when our own hands or our own respiratory muscles could have been used instantly to coax or force air containing oxygen into the victim's lungs.

It is possible, as demonstrated by Robert Hooke in the seventeenth century, and popularized by Meltzer and Aner in 1909,⁹ to perform "respiration without respiratory movement." This means that if the deeper air spaces are flushed with a continuous stream of air or oxygen, an adequate tension of oxygen can be maintained in the alveoli, and the carbon dioxide tension can be kept below normal, so that there is insufficient acid stimulus at the respiratory centers to initiate movement. Only under unusual circumstances is such a technique of replacing normal respiration thought advisable in present-day practice. If sudden respiratory arrest is encountered in a viable patient, good practice

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dictates that we should initiate rhythmic exchange of alveolar atmosphere at once by the most available method at our immediate command.

TECHNICAL CONSIDERATIONS

Normal breathing while at rest is accomplished by intermittent exaggeration of the subatmospheric intrapleural pressure. It is brought about chiefly by synchronous contraction of the intercostal muscles and the diaphragm. Under stress normal breathing is probably a combination of active alternate increase and decrease of pleural pressures. When respiratory muscles cease activity, the lungs are held partly filled at atmospheric pressure by the negative pressure in the pleural cavities, in spite of the elasticity of lung tissue which is tending to collapse the lungs and force the contained atmosphere out. To maintain rhythmic exchange of the atmosphere in the alveoli, three physical changes and combinations of these are available:

- I. Intermittent exaggeration of negative intrapleural pressure.
- II. Intermittent increase of pressure in alveolar spaces.
- III. Intermittent decrease of negative intrapleural pressure.

I. *Intermittent Exaggeration of Subatmospheric Pressure.*—Silvester in 1858¹⁰ described a manual maneuver which accomplished artificial intermittent exaggeration of the negative intrapleural pressure in a manner simulating the normal. With a pad under the shoulders and the patient in the supine position, the arms are raised over the head elevating the ribs, the pleural negative pressure is increased, and air rushes into the lungs. When the arms are returned to the sides, the ribs fall, the pleural pressure becomes less negative, and air rushes out of the lungs. He recommended that the arms be pressed against the ribs as they are returned to the sides to aid in expiration.

Willez previous to 1881,¹¹ and more recently Drinker¹² and others, have popularized mechanical respirators, surrounding the trunk or the whole body below the neck with an airtight box in which the pressure can be intermittently reduced, thus raising the ribs and the abdominal wall and imitating the combined effect of intercostal and diaphragmatic action on the intrapleural pressure (see under III).

II. *Intermittent Increase of Pressure in Alveolar Spaces.*—The intermittent transmission of positive pressure to the alveoli through the air passages was probably the first method of artificial respiration described. Several passages in the Bible have been so interpreted.^{13, 14} Vesalius maintained respiration by this means while he demonstrated the movements of the heart when the anterior thoracic wall of animals had been removed. Intermittent direct inflation of the lungs by hand pressure on a rubber breathing bag attached to a face mask or artificial airway is the common method used by the anesthetist when respiration ceases. He finds it possible to maintain adequate ventilation in this manner.^{15, 16}

Numerous mechanical devices have been constructed to inflate the lungs intermittently. The fireside bellows is said to have been used by Vesalius. Goodwyn in 1786 described an especially made syringe,¹⁷ and every generation since that time has had its favorite for the purpose.

III. *Intermittent Decrease of Negative Intrapleural Pressure.*—To decrease the negative or subnormal pressure in the pleural cavities and thereby cause atmosphere to rush out of the alveoli through the air passages (followed by passive inspiration), the thoracic cage must be made smaller by depressing the ribs or pushing the diaphragm upward, or both. The Schafer prone pressure manual method of artificial respiration¹⁸ typifies the application of this principle. Simple intermittent pressure on the ribs or abdominal wall does the same. Mechanical devices by which the trunk or part of it is inclosed in an airtight rigid container can be used to decrease the negative intrapleural pressure if positive pressure is intermittently applied inside the container.

The combinations of these three principles of maintaining atmospheric exchange are many and varied. Synchronous application of I and II, alternation of II and III, and alternation of I and III have been advocated. Accomplishment is suggested by alternating changes of position of the whole body, by manual manipulation of various parts, and by cumbersome¹⁹ or beautifully intricate²⁰ machines. Hand power, foot power, electric motors, and compressed gas, all have been and are being used to motivate such equipment.

In general, it may be said that manual maneuvers or direct inflation of the lungs from the operator's own respiratory tract have the advantage of instant availability and intimate personal relation to the effort, resulting in the likelihood that the rescuer will more readily appreciate defects in the methods, such as an obstructed airway and inadequate or excessive exchange. The actual method of performing artificial respiration is relatively immaterial. In circumstances where the respiratory tract is free of contaminating material, direct inflation of the lungs by blowing intermittently through the patient's mouth or nose is probably the most readily available. The oxygen cylinder, breathing bag, and mask ought to be available in hospitals and emergency kits. The Silvester method is as efficient as other manual methods.²¹ If the tract is contaminated by foreign material, as after vomiting or drowning, and suction apparatus is not available, the Schafer prone pressure maneuver is safer. The instant application of some method of intermittent exchange, when natural effort ceases, is all important.

Mechanical apparatus is constructed partially of rubber which deteriorates, of intricate machinery which gets out of order, of weighty substances which are difficult to transport. Delay in the application of apparatus is the rule, not the exception. If resuscitation is attempted with apparatus as promptly and as intelligently as with manual maneuvers or direct inflation, the result may be as good, but not better. The enrichment of the atmosphere with oxygen is valuable in either circumstance, and trauma or overventilation,²² as well as inadequate atmospheric exchange, may result from our efforts. A mechanical respirator is as safe and as efficient as the physiologic intelligence and technical skill of the person who operates it and no more so. Comprehension of the physiologic factors involved and intelligent direction of the effort by an experienced physician are essential whatever the method employed.

Whether the rhythmic change in intrathoracic pressure experienced in normal breathing has an important influence upon blood flow has never been

satisfactorily determined. In desperate circumstances intermittent high pressure (40 to 60 mm. of mercury) chest inflation in performing artificial respiration with oxygen has been observed to propel blood through the vessels.²³ Whether the usual rational efforts at performing artificial respiration may aid in maintaining an adequate blood flow has not been determined. Clinical experience leads me to believe that pulmonary circulation, at least, is influenced by rhythmic filling and emptying of the lungs. Attempts to substitute "respiration without respiratory movement" during open chest operations have been associated, after a period of fifteen or twenty minutes, with circulatory depression that appeared to be dependent upon the absence of the movement of the lungs, since circulation seemed to improve when intermittent inflation was resumed.

RESUSCITATION AND THE PHYSICIAN

It is obvious that there are many maneuvers capable of maintaining satisfactory exchange of the alveolar atmosphere when normal respiration ceases. Two factors more important than the method by which exchange is brought about are (a) the promptness with which the artificial method is initiated after normal activity has ceased; (b) the intelligence and physiologic understanding with which the method is applied. Because of a realization of the necessity for promptness, lay rescue squads were instituted and trained to function in the absence of a physician until one could reach the patient. They have served a useful purpose, but, as a result, the medical profession and the public have come to look upon "resuscitation" as a nonmedical effort. Medical schools neglect to teach the clinical application of physiologic principles to the care of acute respiratory morbidity. Many young physicians come to their internships dependent solely upon such knowledge and skill as they have gained from their Boy Scout training. Practitioners of medicine rush to the telephone to call rescue squads from the police and fire departments while patients die who could be rescued. To the disgrace of present-day medical education, sudden failure of respiration is not a rare cause of death in the wards and operating rooms of the modern hospital.

Medical training must include knowledge and skill in the rapid re-establishment of a free and open airway. Specific instruction in the anatomy of the air passages, as it applies to the transfer of atmosphere to and from the alveoli, the use of a simple laryngoscope—its advantages and dangers—the nontraumatic insertion of an artificial airway, the use of suction or gravity to remove contaminating foreign substances, and the physiologic principles of oxygen and carbon dioxide transport, all these as well as methods of artificial respiration, must be included in the training of a physician. Equipped with such knowledge and skill, the physician will instinctively do the right thing in a moment of emergency. Clearing the airways and accomplishment of exchange will be prompt because they are instinctive. Facility in the care of respiratory failure can be taught by the department of anesthesiology. Anesthetized patients can be used as demonstration material to the advantage of patients as well as students. If experience is acquired when no serious emergency exists, the loss of time and waste motion characteristic of excited persons in an emergency may be avoided.

SUMMARY

"Resuscitation" is not the resurrection of the dead, but the re-establishment of oxygen transport from atmosphere to body tissues when either factor in that transport has been suddenly interrupted.

The restoration of circulation, once it ceases, is rarely possible. Treatment directed at the heart and blood vessels other than the injection of blood and other fluids is indicated only in unusual circumstances.

The most logical procedure in emergencies demanding resuscitation is the provision of (a) a free and unobstructed airway; and (b) intermittent exchange of alveolar atmosphere containing excess oxygen.

Mechanical devices will rarely be needed if instant intelligent aid is at hand. They do not produce more adequate exchange than direct inflation from the operator's own lungs or manual maneuvers, nor higher concentration of oxygen than a simple oxygen cylinder, breathing bag and mask, and they may over-ventilate or traumatize the lungs. Their sole advantage is seen in cases requiring long periods of artificial respiration, such as drug poisoning, paralyses, and the like, when the operator might become exhausted. Rarely will such cases come under the classification of "resuscitation".

Finally, the re-establishment of the physiologic functions which transport oxygen is within the physician's province. The fact that some doctors even in hospital practice are dependent on lay rescue squads is a reflection on medical education.

REFERENCES

1. Courville, Cyril B.: Asphyxia as a Consequence of Nitrous Oxide Anesthesia, *Medicine* 15: 129, 1936.
2. Schreiber, Frederic: Cerebral Anoxia and Anesthesia, *J. Michigan M. Soc.* 38: 1050, 1939. *Ibid*: Mental Deficiency From Paranatal Asphyxia, *Proc. Am. A. Ment. Deficiency* 41: 95, 1939.
3. Fisher, Hart Ellis: The Evolution of Resuscitation, *Trans. 20th Annual Safety Congress Nat. Safety Council*, 1932.
4. Snow, John: On Chloroform and Other Anesthetics, London, 1858, J. Churchill, pages 261-262.
5. Hyman, A. S.: Resuscitation of Stopped Heart by Intracardiac Therapy. II. Experimental Use of Artificial Pacemaker, *Arch. Int. Med.* 50: 283, 1932.
6. Beck, C. S., and Mautz, F. R.: The Control of the Heart Beat by the Surgeon, With Special Reference to Ventricular Fibrillation Occurring During Operation, *Ann. Surg.* 106: 525, 1937.
7. Singh, Indeyit: Absorption of Oxygen From the Subcutaneous Tissues, *Quart. J. Exper. Physiol.* 22: 193, 1932.
8. Tummliffe, F. W., and Stebbing, G. F.: The Intravenous Injection of Oxygen Gas as a Therapeutic Measure, *Lancet* 2: 321, 1916.
9. Meltzer, S. J., and Auer, J.: Continuous Respiration Without Respiratory Movements, *J. Exper. Med.* 11: 622, 1909.
10. Silvester, Henry R.: A New Method of Resuscitating Still-Born Children, and of Restoring Persons Apparently Drowned or Dead, *Brit. M. J.* July 17, 1858, p. 576.
11. Woillez, E.: Nouveau Spirophore (Original presented in 1876), *Bull. Acad. de m d. Paris* 10: 847, 1881.
12. Drinker, P., and Shaw, L. A.: An Apparatus for Prolonged Administration of Artificial Respiration, *J. Clin. Investigation* 7: 229, 1929.
13. Genesis 2: 7.
14. II Kings 4: 32-38.
15. Guedel, A. E., and Treweek, D. N.: Ether Apnoeas, *Anesth. & Analg.* 13: 263, 1934.
16. Waters, R. M.: Carbon Dioxide Absorption From Anaesthetic Atmospheres, *Proc. Roy. Soc. Med.* 30: 1, 1936.
17. Goodwyn, Edmund: The Connexion of Life With Respiration, London, 1788.

18. Schafer, E. D.: The Relative Efficiency of Certain Methods of Performing Artificial Respiration in Man, *Proc. Roy. Soc. Edinburgh*, Part I, 25: 39, 1903-05.
19. Thunberg, T.: Der Barospirator. Ein Apparat für Künstliche Atmung nach einem neuen Prinzip, *Abderholden's Handbuch der biologischen Arbeitsmethoden*, 1927, Abt. 5, Teil 1, 561.
20. Craaford, C.: On the Technique of Pneumonectomy in Man, *Stockholm*, 1938, p. 53.
21. Waters, R. M., and Bennett, J. H.: Comparison of Manual Maneuvers, *Anesth. & Analg.* 15: 151, 1936.
22. SeEVERS, M. H., Stormont, R. T., Hathaway, H. R., and Waters, R. M.: Respiratory Alkalosis During Anesthesia, *J. A. M. A.* 113: 2131, 1939.
23. Waters, R. M.: Resuscitation: Artificial Circulation by Means of Intermittent High Pressure Chest Inflation With Oxygen. A Preliminary Report, *Anesth. & Analg.* Bull. 15 (Oct.), 1921.

CLINICAL PATHOLOGY: PAST, PRESENT, AND FUTURE*

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EVEN the most casual observer must admit that medicine—which includes all that pertains to the recognition, management, and control of disease—is in a constant state of flux. So many, so varied, and so continual have been the advances in the last twenty-five years that the limits of the future may almost be thought to be defined only by the limits of the imagination. Particularly may this be said of that phase of medicine which has come to be known as clinical pathology, at long last officially recognized for what it has long been in actuality—a specialized, and highly specialized phase of the practice of medicine.

No one will deny that the first and most important step toward the solution of a clinical problem is the recognition of its nature. In other words, accurate diagnosis is the essential prerequisite for intelligent treatment.

When disease could be regarded as a manifestation of the wrath of vengeful gods, diagnosis was indeed an easy matter. But as the centuries rolled on, imperceptibly merging the past with the present, human nature became less credulous, and concepts of disease less and less associated with dogmatic formulas and more and more dependent upon attempts at the logical and rationalized interpretation of observed phenomena.

Inevitably it came to be realized that it was not enough to give the disease a name. And as the realization of the necessity for more than this became keener, the more persistent and searching were the endeavors to uncover the underlying, basic mechanism upon which the phenomena of disease depend. Fascinating as is the story of these developments in retrospect, it cannot be told here. For the way was long and devious, the forward steps slow and toilsome, and the history of medical progress, though colorful, cannot be told briefly. Suffice it to say that each advance made more clear the concept upon which modern medicine is based: That just as health, in the last analysis, is a condition characterized by perfect and frictionless functional efficiency, so disease, on the contrary, is the manifestation of disturbance, alteration, or loss of function.

From this concept clinical pathology was derived. For while it is obvious that the skilled physician is characterized by the degree to which he has cultivated his powers of observation as well as—what is equally important—the degree to which he has developed ability to interpret the significance of what he observes, it is equally obvious that there are inescapable limits to what may be ascertained by clinical observation alone.

Observation, which signifies the accumulation of pertinent data by all ordinary means, may well lead, for example, to the clinical suspicion that the patient is a diabetic. But no amount of ordinary observation suffices to demon-

*From the Laboratories, Atlantic City Hospital.

strate the essential, pathognomonic evidence of the disease—hyperglycemia. For this, recourse must be had to the specialized methods of the clinical laboratory; and so there came into being what, for a time, it was customary to speak of as “laboratory medicine” in which the “test” was the thing.

Now while laboratory procedures are best and most properly thought of as specialized phases of the examination of the patient, the early days of “laboratory medicine” present aspects at times suggesting merely an elaboration of an older order exemplified by the urologist of the past who, from his stand in the market place, from a paucity of data drew pontifical prognostications of extraordinary inclusiveness. It seems not altogether unjust to see some faint shadow of his counterpart in the surgeon of old, for whom a white cell and differential count plumbed the depths of scientific medicine, or the clinician of the same day for whom glycosuria and diabetes were synonymous, and albuminuria made the diagnosis of “Bright’s disease.”

Medicine, like all human endeavors, has a tendency to go to extremes. If this so-called early phase of laboratory medicine evidenced a tendency to erect imposing diagnostic structures upon somewhat slender laboratory foundations, the phase that followed was often characterized by a multitude of laboratory reports as *ipso facto* evidence of a prodigal expenditure of all the resources of medical science.

In this streamlined present we are likely to think of those days as very long ago indeed. But it was only in 1924 that MacCarty¹ commented that in a series of 60,645 patients there were recorded 225,785 laboratory tests, an average of over 3 per patient. This shows, MacCarty adds, that “clinicians recognize that the diagnosis of disease is no longer covered by history, inspection, palpation, and auscultation.”

It may be further commented that the series in question comprises cases seen in a well-organized and outstanding clinic and that the laboratory procedures in question may, therefore, well be assumed to have been clearly indicated and wisely chosen as likely to be truly informative. This should be emphasized for it cannot well be denied, and certainly will not be denied by directors of hospital laboratories in general, that such laboratories are not infrequently called upon for examinations not always productive of essentially significant data. In other words, it can safely be said that a definite number of laboratory reports constitute “routine” procedures mainly for record purposes, and that others are neither necessarily wisely chosen, truly indicated, nor likely to be productive of clinically applicable or utilizable information.

If there should be a desire to question this, proof can be found in the requisitions for “blood chemistry,” for “general examination,” and so on, from the experience of almost any clinical pathologist. And if this is true of hospitals where the staff is presumably chosen on the basis of attainments, no comment is required on the “office laboratory” where the technician of varied training reigns supreme even though she may be simply the office attendant trained by the clinician.

Few have been quicker than the clinical pathologist to recognize and decri what, later, clinicians perhaps more loudly and frequently proclaimed: That the clinical value and utility of laboratory procedures is based essentially upon their

critical selection in accordance with the particular clinical problem at hand, and equally essentially upon the intelligent and critical evaluation of their results. It cannot be overemphasized that the methods of the laboratory are essentially specialized phases of the examination of the patient, the purpose of which is to detect and to measure alterations in function, the essential phenomena of disease whose existence, degree, and intensity can seldom be ascertained in any other way.

Once it is appreciated that the clinical value of any laboratory procedure depends, not upon its nature, but upon the interpretation of its results, the inevitability of the evolution of the clinical pathologist becomes apparent. For as the investigations into the essential and interlocking concomitant phenomena of disease embraced a necessarily widening field and developed into more and more complex and inclusive phases of physiology, immunology, serology, biochemistry, and the related phenomena of tissue growth, destruction, and repair, "laboratory medicine" became less and less primarily concerned with methods and technique and more and more focussed upon underlying basic principles and applications.

Inevitably the cloistered pathologist of old, engrossed in the study of the aftermaths of disease as evidenced in the dead, gave way to the *clinical* pathologist whose interest lay in the phenomena of disease and their development and progress in the living patient.

In its early days clinical pathology may well have been regarded as a specialty in which it was relatively easy to become skilled and competent, principally because this was largely an era of technical developments and the interpretation of laboratory procedures seemed relatively simple and well defined. But as the field became more and more inclusive, methods more complicated as well as more numerous and, particularly, their basic principles more intricate, clinical pathology became indeed complex, especially in its implications. Hence the clinical pathologist worthy of the name was forced to become to the limit of his ability an authority upon disease and its varied phenomena.

The clinical pathologist of today, therefore, is not, nor can he be, defined—as once was rather widely held—as an individual characterized mainly by manipulative dexterity. On the contrary, many an outstanding clinical pathologist would be the first to admit that in purely technical aptitude he may well be outstripped by many of his technical assistants. His place in modern medicine and his value to it rest not upon his ability to "make tests," but rather upon his ability to indicate, first, which avenues of laboratory approach may best be suited to the particular clinical problem, and, second, how the phenomena demonstrated by their results may best be interpreted in the light of all available data. Hence the term "*clinical* pathologist," for clinical training and clinical experience are essential. While he may learn technical minutia from books, just as he may learn signs and symptoms from books, he must learn medicine—of which clinical pathology is a phase comprehensive in its implications—at the bedside from a study of the patient.

That clinical pathology in its most desirable aspects represents a coordination of the clinical and laboratory aspects of disease is exemplified by the fact that outstanding surgeons and clinicians not infrequently have served an ap-

strate the essential, pathognomonic evidence of the disease—hyperglycemia. For this, recourse must be had to the specialized methods of the clinical laboratory; and so there came into being what, for a time, it was customary to speak of as “laboratory medicine” in which the “test” was the thing.

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Medicine, like all human endeavors, has a tendency to go to extremes. In this so-called early phase of laboratory medicine evidenced a tendency to erect imposing diagnostic structures upon somewhat slender laboratory foundations, the phase that followed was often characterized by a multitude of laboratory reports as *ipso facto* evidence of a prodigal expenditure of all the resources of medical science.

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intervening period there was much to indicate that the trend of clinical pathology was—if slowly—moving toward the ideal described.

If, on the one hand, there are more and more hospital laboratory directors whose functions are truly those of coordinators, liaison officers between the laboratory and the wards, on the other, there are still hospital laboratory directors, physicians, who are denied a place on the medical staff; and others—many others—who are not physicians and whose training has been essentially purely technical.

Can the present trend continue? What will be the trend of the next quarter of a century? He would be brave indeed who would attempt to answer these questions with any assumption of finality. It is well, however, to take stock of the situation and to at least marshal the facts which may well govern it in the future.

It is a matter of fact and record that there are not at present enough competent and accredited clinical pathologists to go around. This is the reason why it is necessary for hospitals to skirt approval, so to speak, by the not always entirely satisfactory expedient of the part-time pathologist shared by several hospitals. It is also the reason for the somewhat anomalous situation in which directors of hospital laboratories are neither physicians nor truly clinical pathologists.

If the present supply of clinical pathologists is insufficient, what are the prospects for the future supply?

Now it will be admitted that for the development of a clinical pathologist as a finished product there must be, first of all, a clinical pathologist in embryo. Like physicians in general, clinical pathologists reach their peak of ability and skill only after long training and experience; and, again like physicians in general, not being immortal, they eventually cease to function. If there is need—as who can doubt?—for maintaining the strain, not to mention the desirability of increasing its numbers, they must be replaced as they wear out, and such replacement can come only from those who follow willingly in their footsteps.

It is food for thought that in these United States there are less than 500 clinical pathologists certified as such, by the American Board of Pathology; less than 1,000 on the rolls of the American Society of Clinical Pathologists; and very probably not more than 2,000 in all engaged, with varying degrees of propriety, in this field of medical practice. What provision is there to balance the inevitable decrease in these numbers arising from the fact that flesh is mortal?

It may be admitted, indeed, in these days when the economic aspects of medical practice are so constantly, vociferously, and at times even acrimoniously discussed—it is being generally admitted—that the practice of medicine has its business aspects. Certainly, if there are to be doctors, they and their families must be fed, housed, and clothed. This is equally true of those physicians who have chosen to be clinical pathologists.

If it can be believed, as it may be, that “the laborer is worthy of his hire,” it may well be pertinent to speculate upon the relation of economic aspects to the clinical pathologists of the future. For it cannot be denied that in some

measure these will be a factor in determining the future supply of clinical pathologists. And, if it is true that the trend of the present may, to some degree, forecast the trend of the future, this may well be pondered upon.

It seems entirely probable that the clinical pathologist practicing this specialty as a private individual will be eventually, if he is not now, a thing of the past. This assumption may be supported by various factors of which the following are not the least important: (a) The gradual invasion of the diagnostic and even the therapeutic phases of medical practice by state boards and departments of health under the aegis, and with the full encouragement and financial support of the Federal government. (b) The even more aggressive invasion of medical practice in all its phases by the programs formulated by the United States Public Health Service under its Surgeon-General. (c) The increasing invasion of medical practice by hospitals and other corporate bodies in connection with "hospital insurance" and "sickness insurance" plans.

While these may be regarded as the spearhead of the attack, among the auxiliary forces contributing to the extinction of clinical pathology as a phase of the private practice of medicine may be noted: (a) the "diagnostic" laboratory operated by the trained technician; (b) the purely commercially owned and operated "clinical" and/or "diagnostic" laboratory not infrequently attracting "business" by discounts, and so on; (c) the drugstore laboratory doing urinalyses with the help of the directions given in the pharmacopeia.

It could be truthfully said that these auxiliary forces could not exist nor survive except for the support given them by the practicing physician in general. But it is of little avail to point this out. If commercial laboratories suffer from attrition, it will result from the drain of the "office laboratory" which has numerically increased by leaps and bounds since the depression for very obvious, and in the main purely economic, reasons.

In the face of these facts the reluctance of young physicians to enter the field of clinical pathology is readily explainable, for at least one of the reasons for engaging in the practice of medicine is the belief that it offers chance of satisfactory remuneration. While this may come deservedly to the clinical pathologist who has achieved through his attainments the status of a coordinator, correlator, and consultant, or to him who becomes a specialist within a specialty—as the tissue or hematologic diagnostician—these must be preceded by long years of training. Under present circumstances it is hardly strange that small numbers are entering into the field of clinical pathology. It is somewhat strange that clinical medicine can observe with only little apparent interest, if not with equanimity at least without outward perturbation, the gradual disappearance of a phase of medical practice by attrition through attack on all fronts; it is strange because what can happen to any one specialty in medicine can also happen in greater or lesser degree to any other specialty as an individualized endeavor.

It is at times even painfully apparent that clinicians, for one reason or another, have shown some tendency to regard the problems of clinical pathology with apathy. It is also true, and may well become equally painfully apparent, that the problems of medicine as a whole cannot be divided into the problems of separate specialties. For just as the specialist cannot safely regard the

patient as consisting only of the particular part, organ, or function with which the specialty itself is mainly concerned, but must see him as a coordinated, interlocking whole, so the physician must look upon medicine as a whole and appreciate that what affects it in part may well have repercussions on the whole.

There are, without doubt, many reasons, some of them not easily defined, for the separation of clinical pathology from clinical medicine. It may be due in part to persistence of its association in many minds with its purely technical aspects; in part, it may arise from the very complexity of the subject which tends to make men think, not of clinical pathologists, but of immunologists, serologists, biochemists, bacteriologists, tissue diagnosticians, and so on, as if all these must necessarily be separate specialties, whereas, in present days, the clinical pathologist—if he is to play his proper part in the understanding of disease—must be oriented in all of these.

It must be admitted too, that clinical pathologists have not always been in the fore in emphasizing the true status of their specialty and their own status in the field of medical practice. The clinical pathologist, it is true, must by his deeds and his accomplishments demonstrate his worth in the general field of medical practice and, having done so, is entitled to receive—and, if necessary, should demand—the same professional status accorded to the specialist in any field.

As Inglis³ has recently reiterated: "A well-trained pathologist endowed with the true scientific spirit, one who can win the confidence of his clinical brethren, will prove to be a boon to any group of practitioners. In competition with none, he can, in his special field, be adviser to them all. If he is of the right calibre he will bring an interest into their work such as they never dreamed of, and, should there be some who feel overburdened with monotonous routine, he may save them from becoming mere carpenters and pillmongers of the profession. To do all this a man must be endowed with high qualities and that is why those who are devoted to pathology hope to attract to its ranks the very best of our graduates."

It is well that clinicians at large should be reminded of these facts, for clinical pathology is an essential phase of clinical medicine. There is today no escape from this important and pertinent fact.

There has been much discussion of the present trend toward the so-called "socialization of medicine." But there has been little apparent interest in, and still less apparent appreciation of, the fact that the socialization of clinical pathology is well on the way if, indeed, it is not already here in appreciable measure. Let it be noted well that when the socialization of one phase of medical practice is fully and openly accomplished, the socialization of others will inevitably follow.

Clinical medicine will do well to regard the present trend of clinical pathology with somewhat less lethargy and equanimity than is customary. It may not be altogether the part of wisdom to ignore the present problem of supply and demand in the field of pathology, to leave its solution to the future, or to accept as a solution the influx of the emigrée—unless, of course, it is felt that such an influx is an easy and acceptable answer to the problem of medical supply and demand.

Unless men of proper caliber can be induced to enter the field of clinical pathology, to spend the time required for prolonged and essential training, medicine in general will suffer, and hospitals—which must be looked upon as centers of post-graduate training—will be increasingly unable to fulfil the function and the destiny which are properly theirs.

It should be frankly said, as Inglis³ has said, “to choose for responsible pathological appointments refugee doctors with inadequate special experience, because they are willing to accept the remuneration and status of technicians, would be unwise.”

The future of clinical pathology and, indeed, the future of medicine itself, may well be said to rest on the lap of the gods and of a Delphian oracle to interpret or to prophesy there is no sign.

REFERENCES

1. MacCarty, W. C.: The Pathologist of the Twentieth Century, *J. LAB. & CLIN. MED.* 9: 365, 1924.
2. MacCarty, W. C.: The Pathologist of 1940, *J. LAB. & CLIN. MED.* 9: 733, 1924.
3. Inglis, K.: Pathology in Practice, *M. J. Australia* 1: 609, 1940.

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PROGRESS

TUBERCULOSIS—PRESENT AND FUTURE

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IN MOST parts of the world where statistics have been kept the death rate from tuberculosis has been steadily declining for about one hundred years.

About 1780 the estimated death rate from consumption in England was 650 per 100,000 living inhabitants.

It is expected that the death rate in the United States will fall below 45 per 100,000 in 1940. According to Dublin, this will represent a decline of 37 per cent in the last ten years, and a decline of 61 per cent in the last twenty years. In forty years the death rate has dropped 76 per cent.

It is probable that tuberculosis has waxed and waned in different countries during the world's history. Historical studies suggest that an epidemiologic period may run over two hundred years.

In London there was a high death rate in 1650 which then declined and rose again to a high peak in 1780.

We are now approaching the lower level of a declining cycle and with the enlightenment which is ours today a new cycle should never begin. It is curious that in studying the present decline, which is common to most countries where statistics are available, that this decline has been almost continuous and that the discovery of the bacillus in 1882, the increasing isolation of the tuberculous in sanatoriums since 1900, and the increase in collapse therapy since 1912, have not definitely accelerated the decline.

In this country the problem is still a serious one among the colored population and among the Mexican residents. In spite of the excellent work of clinics and agencies, many "carriers" of the disease continue to be undiscovered.

Relapse is still too frequent and this is often the result of too short a sanatorium regime as well as lack of aftercare. The time will come when every open case of tuberculosis will be segregated. This will mean that many States must increase their sanatorium beds. Approximately 600,000 individuals in this country have active tuberculosis. Only 90,000 sanatorium beds are available when there should be 150,000.

Thanks to the Department of Agriculture bovine tuberculosis is being rapidly eliminated. The more general pasteurization of milk has greatly lessened human infection by the bovine bacillus.

Teaching the detection, control, and eradication of tuberculosis should begin in the public schools. Children should be taken into the fields to study Nature and to be shown the parasitic diseases of the flora and fauna. With such beginnings the problem of human disease will be more easily imparted to them.

The detection of childhood tuberculosis by the increasing application of the intradermal skin test with the purified protein derivative has been a great advance in preventive medicine. Reactors can be carefully watched and their young lives can be controlled. In many communities Nutrition Camps have been established, and these are ideal for children who are below par or who react to tuberculin.

More and more x-ray examinations of young adults and children are employed; these examinations should be repeated at intervals on any person suspected of having the disease. The use of miniature films in surveys of large groups is of increasing value.

Since clinical and biographical studies show tuberculosis is frequently a family disease, physicians who attend such families have great responsibility.

For many years the trend of treatment has been against climate and also against the long rest regime, although both factors have been of definite value.

Since 1912 various forms of collapse therapy have been introduced. Many physicians feel such therapy should be instituted as soon as the diagnosis of adult pulmonary tuberculosis has been made.

Added to artificial pneumothorax we have thoracoplasty, phrenic nerve crushing, extrapleural pneumothorax, and other surgical procedures which now include the Monaldi procedure of suction aspiration of tuberculous cavities. It is possible the pendulum has already swung too far in the surgical direction. Artificial pneumothorax is gradually giving way to more and more thoracoplastic operations. In spite of the help from pneumonolysis too many results are incomplete and too many complications still occur from pneumothorax treatment. The length of treatment is also a handicap.

While physical examination still plays an important part in diagnosis and study, the x-ray has largely supplanted this and is of great value in guiding pneumothorax treatment. Laminagraphy is now adding more important information.

The present sees an increase in the use of the bronchoscope for the diagnosis of bronchial lesions and their treatment, for the detection of strictures when weighing thoracoplasty, and for final proof that sputum is negative.

In research laboratories the chemistry of the tubercle bacillus is being carefully studied, and what is of probably more importance the growth factors are being discovered.

All efforts to discover a chemotherapy treatment have failed. Numerous laboratories have investigated the value of sulfanilamide, of sulfapyridine, and other derivatives of these drugs, but no success has been obtained.

The increase of blood platelets in active and chronic tuberculosis has never been explained by laboratory workers.

Clinicians note "leads" which may be followed. Why, in patients with diabetes, who become infected, does the tubercle bacillus progress so rapidly? Why do certain families, who are prone to high blood pressure and to nephritis, seem so immune to tubercle bacillus destruction?

Clinicians have observed that in tuberculous families the children who resemble in constitution a parent who has belonged to the high blood pressure group have escaped serious infection when the children of the tuberculous parent's type died.

Why is there a lessened incidence of pulmonary tuberculosis in patients who develop hyperthyroidism?

Vaccination by B.C.G. (*Bacillus Calmette Guérin*) has gained in usage in Europe, and laboratories in this country have established some value in animals, especially with intravenous administration.

With the continued decline in the death rate a form of vaccination may not be necessary. Shortage of food may again increase the number of tuberculous victims on the continent of Europe. The improved housing and living conditions in the United States, together with the activities of the National and State Tuberculosis Associations, should assuredly prevent another serious rise in the tuberculosis death rate in this country.

CLINICAL AND EXPERIMENTAL

DISSECTING ANEURYSM OF THE AORTA*

CASE REPORT

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THE relative infrequency with which the diagnosis of dissecting aneurysm of the aorta is made ante mortem has prompted us to report a case observed on the wards of the Edward J. Meyer Memorial Hospital. For many years this disease has been known to pathologists, but until recently the clinical observations have been few and the diagnosis has been made at post mortem. The true explanation of this probably lies in the fact that the disease is often confused with various intrathoracic or intra-abdominal conditions which it closely simulates. The relative infrequency of the ante-mortem diagnosis is more evident when it is realized that only twenty-five cases can be found reported in the literature.¹ The disease itself occurs predominantly in males between the ages of 40 and 60 years, in whom there is a usual history of hypertension. There is no pattern to be followed in the diagnosis, but certain features when present must be analyzed in proper combinations to eventuate the correct diagnosis.² These include: (1) the sudden onset of pain in a person with hypertension. This pain not infrequently radiates to other portions of the body but rarely the arms; (2) the presence of a rapid, enlarged heart with or without murmurs; (3) the presence of shock; (4) slight fever and leucocytosis; (5) dyspnea and cyanosis; (6) variation in the pulses between the upper and lower extremities.

There have been many theories advanced as to the cause of the disease, and the division into predisposing and exciting factors has been advocated.³ The predisposing factors include:⁴ (1) Virchow's view that most of these aneurysms arise on the basis of atheromatous ulcers. The fact that arteriosclerosis was not observed in some of the cases at necropsy makes this a tenable explanation in only a few. (2) Inflammation which may weaken the wall of the aorta sufficiently to predispose it to rupture. (3) Medial degeneration of the aorta, in which there is focal hyaline degeneration resulting in cyst formation, has recently been recognized as strongly predisposing to the disease.

With the presence of a predisposing factor, however, the problem of what is the actual causative factor presents itself. Here it has been agreed that two primary factors play a part: (1) physical trauma, and (2) internal trauma due to either emotion or exertion.

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Pathologically⁵ the aorta usually shows an irregular tear which extends through the interior and partly through the media into which blood penetrates until it finds a plane of cleavage and begins to dissect. The blood enclosed in this sheath composed only of adventitia and media most often perforates at some point.

Clinically, as already indicated, certain features suggest the diagnosis. The patients are acutely ill and fail progressively, and the ultimate prognosis is poor, with death occurring in from a few hours to several days. In the differential diagnosis (1) coronary thrombosis, (2) embolism, (3) abdominal catastrophe, and (4) pneumonia are most frequently confused with the diagnosis.



Fig. 1.—A, The large oval represents the hemorrhagic mass over the right auricle. It extends around the base of the heart and aorta with compression. B, the fibrous tag on the pericardium where a similar large amount of clot was found partly organized; C, the dissecting hemorrhage above, in between the coats, and below outside of aorta continuous with hemorrhage A.

CASE REPORT

CASE 379362.—The patient was a white male, aged 51 years, whose occupation was a fireman. He was referred to the hospital on December 5, 1938, with a history of eight hours' duration. He complained of being awakened from his sleep by a sharp, excruciating pain, located diffusely throughout the lower abdomen, but more pronounced about the umbilicus. This pain radiated through to the back, and at the onset was accompanied by emesis on two occasions. No blood was present in the emesis, and the stools were normal in color and consistency. His past history revealed that he first sought medical attention in January, 1937, at which time he was admitted to the diagnostic clinic. His history at that time was of precordial pain of three years' duration brought on by exercise. This pain occurred about one to two times monthly; it lasted one to two minutes and was accompanied by dyspnea, palpitation, and weakness. He also gave some history of occasional frontal headaches, poor vision, and nocturia two to five times. He denied past illness, except for urethritis at the age of 28. At this time (January, 1937) his physical findings revealed the following positive features:

Eyes: Pupils equal, regular, and reacted to light and accommodation.
Mouth: Dental caries.

Chest: Resonant and clear throughout.

Heart: Enlarged, regular, good tone, precordial systolic bruit, aortic second sound accentuated.

Blood pressure: 220/120—Vessels moderately firm.

Abdomen: Negative except for a small left inguinal hernia.

Extremities: No edema—reflexes normal.

His Wassermann test was negative and his electrocardiogram showed an occasional extraventricular systole with slurring QRS. He was advised to return for further therapy but failed to do so. The next association with him was at the time of his present admission. When admitted, he appeared to be well developed and well nourished but seemed to be suffering much pain. He was breathing about thirty times per minute but showed no cyanosis. The positive findings were as follows:

Chest: Elliptical in shape. Expansion somewhat limited on the right side. Resonance was unimpaired. The breath sounds were somewhat suppressed in the right base, otherwise vesicular throughout. No râles were heard.

Heart: Enlarged to the left, regular in rate, good tone. Aortic second sound accentuated. Blood pressure 220/124.

Abdomen: Moved with respiration. There was resistance to palpation in the right upper quadrant. Tenderness was present throughout, more so in the gall bladder region. No organs or masses were felt.

Extremities: No edema.

The impression at the time of admission was (1) hypertension with myocardial hypertrophy and dilatation, (2) possible cardiovascular syphilis and aneurysm, and (3) possible surgical abdomen.

Because of the possibility of an acute abdomen, surgical consultation was obtained. In addition to the findings already mentioned, the patient was now complaining of hemoptysis and pain in the chest on deep inspiration. His urine was negative and his white blood cell count was 22,250, with 92 per cent polymorphonuclear cells. The possibilities entertained at this time were (1) perforated ulcer, (2) coronary thrombosis, and (3) acute pancreatitis.

While no definite diagnosis was made, it was thought advisable to observe the patient on the surgical service. Approximately six hours after his transfer to the surgical service, we again saw him. The pain was still present in the abdomen, and the cough, productive of bright blood, persisted. The temperature which was 97.2° F. on admission was now 101.2° F., and the pulse had become more rapid, 88 to 136. The blood pressure which had been 200/104 on admission was now 140/92 on the left arm, and 170/100 on the right arm. His physical findings at this time showed:

Chest: Marked diminution of breath sounds in the right base.

Heart: Enlarged, regular in rate, tones moderately suppressed, a "G" string type of pericardial friction rub was heard in the fifth interspace to the left of the sternum.

Abdomen: There were fullness and spasm with definite tenderness in the right upper quadrant. No masses were palpable.

Extremities: Slight cyanosis of the nails.

The impression at this time was possible coronary thrombosis or dissecting aneurysm.

An electrocardiogram was taken which revealed left axis deviation and myocardial damage. X-ray at this time showed an enlarged heart with the aorta normal. Therapy consisted of opium sedation, rest, and observation. X-ray was repeated on December 20, 1938, and the heart showed an increase in diameter of fully 4 cm. On December 30 fluid was present in the left pleural space, and thoracentesis was performed with the removal of 300 c.c. of straw-colored fluid which microscopically revealed red blood cells, some granular debris, some polymorphonuclear cells with a predominant small round cell element. Although his treatment was symptomatic, the patient did not respond and death occurred on January 11, 1939.

Post-Mortem Findings.—The left pleural cavity contained 1,400 c.c. of a rather thin, clear, yellowish-brown exudate. This contained numerous clots and fibrinous tags, composed of yellowish-green material. The lungs were dark slate gray in color, with a black over-mottling of anthracotic pigment. There were patchy areas where the lung was collapsed; these were firm on section and dark hemorrhagic in color. The parietal and visceral pleurae of the left side were fibrously thickened. Crepitation was present throughout both lungs. The cut surface was dark purplish red color and on compression oozed a large amount of sero-sanguineous frothy fluid. The lymph nodes at the hilus of the lung were enlarged and on section were black in color. The trachea-bronchial tree showed the mucous membrane covered with a marked amount of mucus.

Heart: The heart was markedly enlarged. The pericardium was adherent. On section through into the heart, a large, currant jellylike clot about the size of an orange, was found anterior to and somewhat to the right of the right ventricle in the pericardial space. This bulged considerably, making the heart appear large laterally. The aorta was split into two layers, this split extending to the base of the heart where the pericardium was attached around the aorta. There was a large amount of dark clotted blood between these layers. This space communicated with the large pericardial space filled with blood. The myocardium was of fair tone. The valves and valvular orifices were clear. The septum left ventricle beneath the cusps revealed subendocardial hemorrhage. The aorta showed numerous thickened areas of raised yellowish plaques. One of these communicated with the split space. The pulmonary arteries and veins were clear.

Abdomen: Abdominal viscera were essentially normal.

Anatomic Diagnosis.—Aortal arteriosclerosis with dissection between the coats and rupturing into a pericardial pocket, a space otherwise obliterated by fibrous adhesion, was found.

CONCLUSION

1. A dissecting aneurysm of the aorta, proved anatomically, has been presented.
2. The difficulty of clinical diagnosis is emphasized.

REFERENCES

1. Claiborne, T. S., and Haller, Emory D.: Dissecting Aneurysm of the Aorta: Antemortem Diagnosis and Course of Fifty-Three Days. Case Report, *Am. Heart J.* 15: 358, 1938.
2. Hamburger, Morton, Jr., and Ferris, Eugene B., Jr.: Dissecting Aneurysm. A Study of Six Recent Cases, *Am. Heart J.* 16: 1, 1938.
3. Tyson, D. Dawson: Dissecting Aneurysms, *Am. J. Path.* 7: 581, 1931.
4. Glendy, R. E., and Castleman, B., and White, P. D.: Dissecting Aneurysm of the Aorta, *Am. Heart J.* 13: 129, 1937.
5. Boyd, William: Textbook of Pathology, Philadelphia, 1938, Lea & Febiger, p. 445.

A NOTE ON *SACCHAROMYCES FRAGILIS* JORGENSEN ASSOCIATED WITH PATHOLOGIC CONDITIONS IN HUMAN BEINGS*

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SACCHAROMYCES FRAGILIS was first isolated by Jorgensen from kefir, and has repeatedly been found in dairy products. Within the last fifteen years, there have been two reports of the isolation of yeastlike fungi from pathologic conditions in human beings in which the organisms proved to be *Saccharomyces fragilis* Jorgensen. In both cases, the organisms were first classified as some other species, but upon detailed study at the Centraalbureau voor Schimmelteeltures at Baarn, Holland, they proved to be strains of *Saccharomyces fragilis*. In the first case, Redaelli in 1925 (Stelling-Dekker, 1931) reported the isolation of *Saccharomyces cavernicula* n. sp. from the lung of a tuberculous patient. Of this culture, Stelling-Dekker (1931) writes as follows: "A careful consideration of this species allows one to conclude that all major characteristics agree with those of *Saccharomyces fragilis* Jorgensen. The agreement is so definite that a separation of *Saccharomyces cavernicula* as a variety of *Saccharomyces fragilis* is not warranted. . . ." Another place she writes: "*Saccharomyces cavernicula* Redaelli is identical with *Saccharomyces fragilis* Jorgensen."

In 1935 Ciferri and Redaelli (Diddens and Lodder, 1939) described a species that had been isolated by Careo at Catania from a lesion of the human tonsils and pharynx. It was classified as *Monilia pinoyisimilis* (A. Castellani) Redaelli and Ciferri variety *Citelliana* Redaelli and Ciferri. Of this organism Diddens and Lodder (1939) write: "It evidently belongs to the genus *Saccharomyces* and it is completely identical with *Sacch. fragilis* Jorgensen."

The purpose of this article is to report an organism isolated by Miller (1935) and classified by him as *Monilia pseudotropicalis* Castellani. I secured a transfer of this strain and continued its study and found it to be identical with *Saccharomyces fragilis* Jorgensen (Laffer, 1936). Such results are not surprising for two reasons: first, the difficulty with which some strains are stimulated to produce ascospores; and second, the following statement by Diddens and Lodder (1939): "*Monilia pseudotropicalis* Castellani—*Candida pseudotropicalis* (Castellani) Basgal has to be considered as the imperfect stage of *Saccharomyces fragilis* Jorgensen." The material contained in the following paragraph is taken from the unpublished thesis of Miller (1935).†

Case history. The patient, a male, came to the physician with an acute infection of the tonsils, characterized by a sore throat, fever, malaise, anorexia, fetid breath, terribly coated tongue, headache, backache, and pains in the limbs. White or yellow patches of exudate were present in follicular form. Intensive treatment for follicular tonsillitis in the first seven

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days did not show the slightest improvement. On the eighth day he was given 10,000 units of diphtheria antitoxin, and the dose was repeated on the ninth day; he recovered rapidly without further treatment. At no time were there objective signs of diphtheria.

According to Miller, his culture isolated from this case had the characteristic fermentative reactions of *Monilia pseudotropicalis* Castellani and serologic studies confirmed this relationship. When the unknown strain was used to produce an antiserum, the antiserum reacted with its specific antigen 1:640; with one type culture of *Monilia pseudotropicalis* 1:640; and with a second type culture of *Monilia pseudotropicalis* 1:320. Agglutinin absorption tests substantiated these serologic findings. The usual morphologic studies were not reported in the thesis. In his conclusions Miller stated: "Culture 10Y which produced acid and gas in dextrose, levulose, galactose, sucrose, raffinose lactose, and inulin, corresponds almost exactly to two cultures of *Monilia pseudotropicalis* Castellani (one from New Orleans, Louisiana, and the other from London, England) which fermented the same carbohydrates with acid and gas. These three cultures formed a distinct group by agglutinin absorption."

In my study (Laffer, 1936) of this culture (Miller's 10Y) the cells were characterized as "cells oval and rod shaped, but becoming filamentous in older broth cultures and potato broth, and producing rod shaped to filamentous asci and ovoid to kidney shaped ascospores." The culture produces one to four ascospores per ascus when grown on carrot blocks or on carrot infusion agar for three days or more at room temperature. Careful examination of colonies on malt extract agar (Difco) streak plates showed pseudomycelia penetrating the substrate, with the groupings of the blastospores at the junction of the adjoining elements similar to those produced by the control culture of *Saccharomyces fragilis* Jorgensen, obtained from the Lister Institute (their No. 2303). Cells grown in malt extract broth (Difco) for twenty-four hours were more or less rod-shaped and measured 3.0 to 5.0 microns by 6.0 to 10.0 microns, with a few filamentous cells as long as 12.0 to 21.0 microns. In older cultures the length and number of the filamentous cells increased.

The biochemical changes produced by the unknown and the control culture were the same, namely: acid and gas in glucose, fructose, galactose, mannose, lactose, and inulin; acid and no gas in raffinose, and neither acid nor gas in arabinose, xylose, rhamnose, maltose, salicin, glycerol, mannitol, dextrin, and starch. For these determinations, nutrient broth base (Difco) plus 1 per cent carbohydrate and bromocresol purple was used. Litmus milk, if incubated at room temperature, became acid with evidence of gas production after a few days, but if incubated at 37° C., the milk was curdled with a renninlike curd. There was no evidence of proteolysis at the end of forty-five days of incubation. When tested by the Hammer and Collins (1935) method, the organism did not hydrolyze fat. No true liquefaction of gelatin was observed at 37° C. after forty-five days of incubation, while "softening" occurred in those cultures incubated at 18° C. to 25° C. for forty-two days. An uninoculated control showed no "softening." The term "softening" is used to indicate a stage in which the medium is soft at room temperature but the gelatin becomes solid upon cooling to 5° C. to 8° C. This term is used in contrast to true liquefaction in

which the medium fails to solidify upon cooling to the above temperatures. While Stelling-Dekker reports that *Saccharomyces fragilis* liquefies gelatin, a difference in the composition of the medium may account for the variation in the degree of utilization of the substrate.

SUMMARY

1. A third case in which *Saccharomyces fragilis* Jorgensen has been isolated from a pathologic condition in human beings is reported.

2. Confirmation of the relationship of *Saccharomyces fragilis* Jorgensen and *Candida* (Monilia) *pseudotropicalis* (Castellani) Basgal, as indicated by Diddens and Lodder (1939), is implied.

3. The desirability of an attempt to further confirm this relationship by serologic studies is suggested.

4. While nothing is contained to indicate that *Saccharomyces fragilis* is the causal agent in the infections of the throat, the finding of this species in throat cultures might prove worthy of a more detailed investigation or might prove to be a lead worthy of following. Careful morphologic studies of yeasts found, including repeated attempts at ascospore determination, and the inoculation of lactose, sucrose, inulin, maltose, litmus milk, and gelatin, would probably suffice for preliminary study and tentative identification.

The wide distribution of this organism is clearly shown. I have found this species in cream obtained in Illinois, and have identified as *Saccharomyces fragilis* cultures received from S. Orla-Jensen, Copenhagen, Denmark (from milk); from the late Dr. Henneberg, Kiel, Germany (from yoghurt); from W. Dorner, Liebefeld-Berne, Switzerland (from sour milk and whey); and from Erivan, Armenia, U. S. S. R. (from matzoon). It is easy to see how such organisms could get into the throat and thus be found in examination of throat cultures.

REFERENCES

- Diddens, H. A., and Lodder, J.: On Some Sporogenous Yeasts and Their Imperfect Stages. *Mycopathologia* 2: 28, 1939.
- Hammer, B. W., and Colliers, M. A.: The Numbers of Lipolytic Bacteria in Various Dairy Products as Determined With Nile Blue Sulfate, Iowa Agricultural Experiment Station Research Bull. No. 169, pp. 73-92, 1934.
- Laffer, N. C.: A Study of Lactose-Fermenting Yeasts. Doctor's Dissertation, University of Illinois, Urbana, Illinois, 1936.
- Miller, W. A.: A Study of Pathogenic Yeasts. Doctor's Dissertation, University of Illinois, Urbana, Illinois, 1935.
- Stelling-Dekker, N. M.: Die Hefesammlung des Centraalbureau voor Schimmelleulturen. Erster Teil. "Die Sporogenen Hefen," Verh. Kon. Akad. v. Wetensch. 28: 1931.

THE ACTION OF EPINEPHRINE INJECTED INTO THE PERICARDIAL SAC*

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WHILE the effect of epinephrine on blood pressure when given intravenously has been widely studied, the effect when given by other routes has not received adequate attention. When given by oral administration, it is usually inactive in normal people. Menninger¹ studied the effect of oral administration in various types of hyperthyroid cases. He gave the patients from 2 to 5 mg. of epinephrine in gelatin capsules and concluded that in some cases epinephrine may produce a marked effect on the circulation with rise of pressure. The effect results from absorption through the gastrointestinal tract and not from the mouth or throat.

Meltzer and Auer² found that the subcutaneous injection of 0.4 to 0.5 c.c. per kilogram of epinephrine has no influence on the blood pressure of a rabbit. Intraperitoneal injection also has no effect on the blood pressure, while 0.3 to 0.4 c.c. given intramuscularly may produce a rise of 40 to 50 mm. The rise is not so sudden as it is after intravenous injection.

Amberg³ previously reported that doses of epinephrine that are fatal when given intravenously do not exert pressor effects in cats, dogs, or rabbits when given subcutaneously, although a very large dose (37 mg. per kilogram) raises the blood pressure in the dog.

Halsey⁴ found that 27 mg. of epinephrine given intramuscularly to a 14 kg. dog raised the pressure 30 mm. (from 140 to 170). In man he found that the oral administration of epinephrine does not change the heart action or blood pressure; when subcutaneously administered, a dose of 0.5 to 1.5 mg., or about 0.5 to 1.5 c.c. of 1:1,000 solution, is uncertain in its effects on the blood pressure but usually causes a slight rise. This same dose, however, given intramuscularly may cause alarming symptoms and a rise of pressure of 90 mm. (110 to over 200). Since some people are sensitive to epinephrine, Halsey recommends that smaller doses be given until the reaction is known.

Grollman⁵ quotes Euler and Liljestrand as having found that the subcutaneous injection of 0.7 mg. of epinephrine in man raised the blood pressure 17 mm. and increased the cardiac output from 4.3 liters to 7.5 liters per minute; this indicated both stimulation of cardiac activity and diminished peripheral resistance.

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Auer and Gates⁶ found that when 0.25 c.c. of epinephrine solution (1:1,000) per kilogram is given intratracheally in rabbits, a rise of pressure occurs after a few seconds and lasts for about five minutes. Jackson⁷ records similar findings. We have found similar results to follow injection into the pericardial sac of dogs (see Figs. 1 and 2).

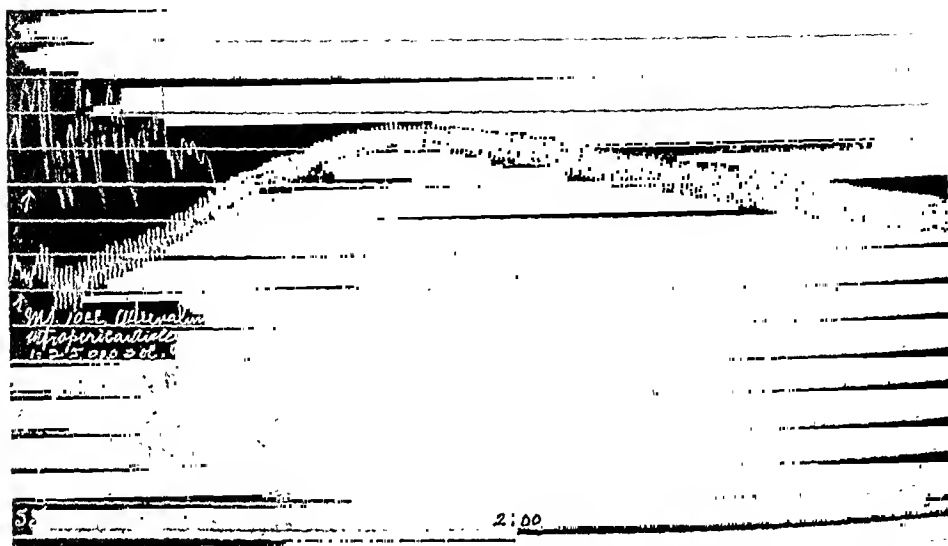


Fig. 1.—The effect of 10 c.c. epinephrine, 1:25,000, in the pericardial sac. (Dog—19 kg.)

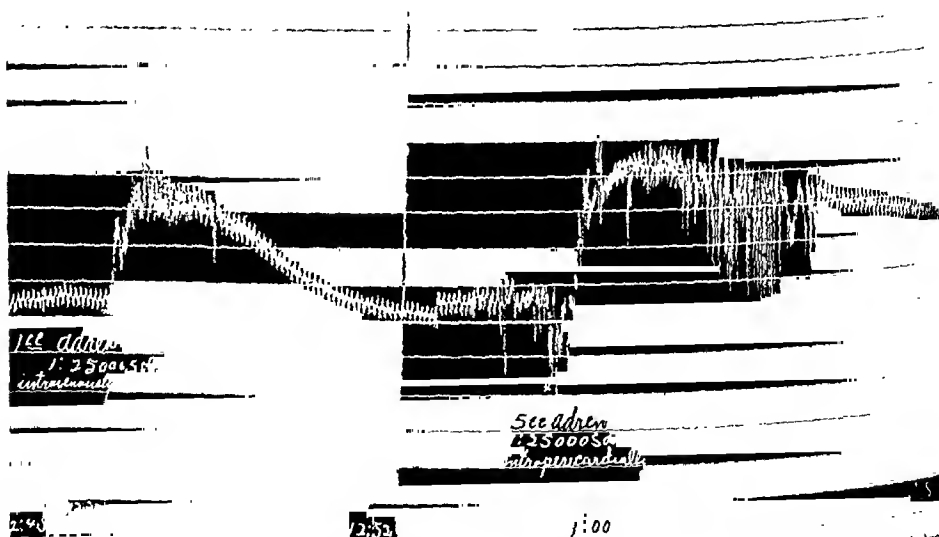


Fig. 2.—The comparative effect on blood pressure of 1 c.c. epinephrine, 1:25,000, intravenously and intrapericardially. The rapid rise in this case after intrapericardial injection is unusual.

METHOD

Dogs were anesthetized with 35 mg. per kilogram of pentobarbital in solution, given intraperitoneally. Blood pressure was recorded from the carotid artery. Artificial respiration was used throughout. The chest was opened and the solution of epinephrine placed in the pericardial sac so that none was lost

by leakage. Duodenal activity was recorded by the balloon method. The tracings show the effect. After and before injection into the pericardial sac, an injection was made into the saphenous vein in order to compare the relative effects of adrenalin when given intrapericardially and intravenously.

CONCLUSIONS

1. The effect of epinephrine injected into the pericardial sac is less abrupt but prolonged about five times as compared to the intravenous injection. About five to ten times the intravenous dose is necessary to produce identical rise in blood pressure.

2. The intrapericardial action of epinephrine in smaller doses is somewhat irregular on the blood pressure but is very constant on the intestines.

REFERENCES

1. Menninger, W. C.: Epinephrine, *J. A. M. A.* 84: 1101, 1925.
2. Meltzer, S. J., and Auer, J.: The Rate of Absorption From Intramuscular Tissue, *J. Exper. Med.* 7: 59, 1905.
3. Amberg, S.: Toxicität des Wirksamen Principis der Nebennieren, *Arch. internat. de pharmacodyn. et de therap.* 11: 57, 1902.
4. Halsey, J. I.: Observations on Adrenalin, *Tr. A. Am. Physicians* 24: 520, 1909.
5. Grollman, A.: The Adrenals, Baltimore, 1936, Williams & Wilkins Co., p. 121.
6. Auer, J., and Gates, F. L.: Some Effects of Adrenalin When Injected Into the Respiratory Tract of Rabbits, *J. Pharmacol. & Exper. Therap.* 6: 608, 1915.
7. Jackson, D.: Experimental Pharmacology, St. Louis, 1939, The C. V. Mosby Co., p. 142.

TORULA INFECTION OF THE CENTRAL NERVOUS SYSTEM*

A REPORT OF A CASE WITH NECROPSY FINDINGS

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SINCE 1907 sporadic reports of torula infection have appeared in the literature. By far the most comprehensive monograph on the subject was presented by Stoddard and Cutler in 1916. In view of the frequency with which this infection is being reported, it is noteworthy to quote verbatim Stoddard's memorandum in 1916: "It would not have been too venturesome to have prophesied that the organism would be found at some future date producing nervous diseases in man. It will take time to determine how common this type of infection is, but many facts indicate the possibility of it being frequently present without recognition."

In 1939 McGrauder published protocols of 3 cases observed at the University of Virginia Hospital since October, 1935, in which he succeeded in isolating the organism. In a personal communication he submitted the following outline:

1. The technician who examines the spinal fluid must be torula conscious.
 2. Identify as a yeast (as contrasted with cells) in the counting chamber.
- The count is made as usual with acetic acid to lake any red blood cells present.

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Torulae might be mistaken for lymphocytes (and usually are) unless one has the probability of torula in mind. The torula organism will show some budding forms, and a thick wall, often surrounded by a definite capsule. No cytoplasm is seen.

3. If torula is suspected, mix a drop of sediment (from centrifuged spinal fluid) with a drop of Greenthal's stain on a slide, cover with a cover glass and examine. The stain will show definitely the nuclei and cytoplasm of the usual cells, distinguishing them clearly from the torulae, which show a definite "cellulose-like" wall, surrounded by a wide purplish or bluish capsule.

4. Confirm by culture. *Torula* usually reproduces by budding only, never forming mycelium, and does not ferment sugar.

It becomes evident that the apparent increase in reported cases is not necessarily an indication of an increased frequency of the infection in man, but rather an indication of an increased interest in the problem with a concurrent improvement in laboratory diagnosis.

The following case report, with its autopsy findings, is as typical of the vagaries of this infection as other cases reported to date.

CASE REPORT

A colored girl, aged 19 years, was first seen on March 3, 1938. She was prostrated, complained of an intractable headache of several weeks' duration, and had photophobia. The examination revealed a marked nuchal rigidity as the only positive sign of any cranial involvement. Ordinary palliative measures were of no avail. After three days, a spinal tap was done for diagnostic purposes as the picture suggested tuberculous meningitis. For the next three days there was no relief. On the assumption that it might be tuberculous meningitis, notwithstanding the negative findings in the spinal fluid, she was placed on a high vitamin D intake, and to my amazement she began to improve almost immediately. She returned to normal activity in two weeks.

Five months later the girl again became ill. In the early days of this recurrence, the picture was identical with that originally presented. This time, however, a series of neurologic signs appeared, following one another in rapid succession. On August 11 she lay in bed in a stupor, with her head and eyes sharply deviated to the right, while the right arm and leg were flaccid. The left hand constantly clutched at the bedclothes. The abdominal reflexes were absent. Both pupils reacted very sluggishly, and the right one was smaller than the left. There were reddening of the fundi, with engorgement of the veins. The jaw was tonically closed. There were positive Brudzinski and bilateral Hoffman signs. The following morning the patient was still in coma. The right limbs remained flaccid and both ankles showed clonus. Her temperature rose to 106° F.; her pulse rate increased to 140 and her respiratory rate remained between 20 and 30. On August 13 she died.

Her skin was dry and cool. Her heart, lungs, and abdomen were essentially negative. Neurologically, she continued to present a continually changing picture with such signs as positive Babinski on the right side and negative Babinski on the left. All four extremities were stiff and spastic. She moved the left side involuntarily but not the right side. The pupils reacted slug-

gishly; the veins dilated to twice the normal caliber. No diagnosis was made, although the usual series of differential diagnoses presented themselves with cerebral abscess as the most likely process.

Spinal Fluid Findings.—August 11, clear, under moderate pressure; cell count, zero; chlorides, 330 mg. per 100 c.c.; glucose, 10 mg. per 100 c.c.; smear lymphocytes, 2; tubercle bacilli, negative; Wassermann, negative; culture, negative. August 13, cell count, polymorphonuclears, 11, lymphocytes, 89; protein, 96; globulin, 2 plus; sugar, 31 mg. per 100 c.c.; chlorides, 715 mg. per 100 c.c.; colloidal gold sol, 1111233100; Wassermann, negative.

Necropsy Findings.—An autopsy was performed soon after death. The brain was examined by the Department of Pathology of the Neurological Institute. The skull was of normal thickness. The dura was moderately adherent to the inner surface of the skull. The brain weighed 1,300 Gm. At the base of the brain, in the interpeduncular fossa, on the inferior surface of the hypothalamus and about the optic chiasm and nerves, the leptomeninges were thickened and grayish with an accumulation of yellowish-gray exudate. On section of the cerebrum, no gross lesions of the parenchyma were noted. The spinal cord leptomeningeal blood vessels were congested, but otherwise the cord appeared normal.

Microscopic examination of the area revealed a proliferation of leptomeningeal cells in the form of fibroblasts. There was a heavy infiltration of the membrane by lymphocytes, monocytes, and plasma cells. There were numerous multinucleated giant cells about focal areas of necrosis.

In places in the necrotic areas, there were pale, translucent, round bodies, with double refractive margins. No structural details could be made out within them; they varied in size from that of lymphocytes to that of monocytes. Some of these bodies were also found within the giant cells. These proved to be *Torula histolytica*. There were also a terminal lobular pneumonia and acute ulceration of the duodenum.

CONCLUSION

A case with necropsy findings of *Torula histolytica* infection of the central nervous system is presented.

REFERENCES

1. Stoddard, James L., and Cutler, Elliot C.: Monographs of the Rockefeller Institute for Medical Research 6: 1916.
2. Shapiro, Louis L., and Neal, Josephine B.: *Torula Meningitis*, Arch. Neurol. & Psychiat. 13: 174, 1925.
3. Firor, W. M.: *Torula Infection of the Central Nervous System*, Internat. S. Digest 2: 3.
4. Wortis, S. Bernard, and Wightman, Henry B.: A Case of *Torula Meningitis*, Bull. New York Acad. Med. 4: 531, 1928.
5. Stone, Willard J., and Sturdivant, B. F.: *Meningo-Encephalitis*, Arch. Int. Med. 44: 560, 1929.
6. Freeman, Walter: *Torula Infection of the Central Nervous System*, J. F. Psychol. u. Neurol. 43: 236, 1931.
7. Johns, F. M., and Attaway, C. L.: *Torula Meningitis*, Am. J. Clin. Path. 3: 459, 1933.
8. Levin, E. A.: *Torula Infection of the Central Nervous System*, Arch. Int. Med. 59: 667, 1937.
9. Greenfield, J. G., Martin, J. P., and Moore, M. T.: *Meningo-encephalitis Due to Cryptococcus meningitidis (Torula Histolytica)*, Lancet 2: 1154, 1938.
10. Magrauder, R. Gregory: A Report of Three Cases of *Torula Infection of the Central Nervous System*, J. LAB. & CLIN. MED. 24: 495, 1939.

CUTANEOUS HYPERSENSITIVITY TO IODINE*

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THE occurrence in certain individuals of a local or contact dermatitis following the application of iodine has been described by many authors. Some^{1,4} speak of the symptoms as toxic in origin; others⁵⁻¹² consider them as manifestations of hypersensitiveness; and two authors¹³ do not indicate their opinion. Except for one instance⁷ of a patient giving a delayed reaction by scratch test to Lugol's solution diluted 1:16 (and not to 10 per cent potassium iodide), confirmatory skin tests have not come to our attention.

Most of the reports on iodine dermatitis are based upon single observations; this indicates the infrequent occurrence of this condition.

Recently a medical student, hypersensitive to iodine, became available for study. It seemed desirable, therefore, to investigate his abnormal reactivity in some detail.

CASE HISTORY

The subject for study, to be referred to hereafter as J. A. B., had no past history of allergy. Family history included a maternal aunt with asthma and a father suffering from migraine. Five years previous (at the age of 18) the patient first noticed dermatitis following application of tincture of iodine. Not long before that he had fallen on a cinder track, and his injury had been treated by a local application of tincture of iodine over a large area on his leg. Since then the application of iodine tincture has regularly caused a local dermatitis confined to the area exposed.

EXPERIMENTAL

A preliminary test confirmed the fact that tincture of iodine (7 per cent iodine, 5 per cent potassium iodide), which gives no reaction on normal controls, produced reddening in our patient within seven hours after the application of one drop to the skin. By the second day blisters had developed, with thickening of the epidermis. These blisters exudated serum for approximately two days and then began to dry up. The site of application could be detected for some time; after a month it was still slightly reddened and covered with scabby scales. A 1:10 dilution of the above tincture in alcohol also produced a similar reaction, though of lesser intensity.

Another series of tests was performed on this patient with a tenth normal iodine solution (1.3 Gm. iodine and 3.0 Gm. potassium iodide in 100 c.c.). Tenth normal iodine solution does not irritate the skin of normal individuals, but in

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this patient it caused a reaction which was, however, less marked than that elicited by the iodine tincture. A few slightly raised, pale pink papules were present at twenty-four hours; they continued to increase in number and intensity of coloring so that after forty-eight hours the whole area affected was covered with pinkish elevations. A 1:10 dilution of tenth normal iodine solution failed to produce a reaction.

Powdered potassium iodide and a 1:1 solution of potassium iodide in distilled water failed to give a positive patch test. On the other hand, 0.5 per cent iodine salve in vaseline (crystals finely ground and mixed with vaseline in a mortar) which contained no potassium iodide, produced a marked delayed reaction. These results are tabulated in Table I.

TABLE I
SENSITIZATION TO IODINE

All recorded readings were made on the day following the test. In the patch tests an amount was used equivalent to the bulk of a pea; in the intracutaneous tests 0.02 c.c. was injected. Figures refer to millimeters.

TYPE OF TEST	SUBJECT	TEST PERFORMED WITH:			
		0.5% IODINE IN VASELINE	TINCTURE: 7% IODINE WITH 5% POTASSIUM IODIDE	5% IODINE IN ALCOHOL	SATURATED POTASSIUM IODIDE SOLUTION
Patch	J. A. B.	Pink, elevated	Pale pink, slightly elevated, spotty; 2 small vesicles	Negative	Negative
	Control 1	Negative	Negative	Negative	Negative
	Control 2	Negative	Negative	Negative	Negative
TYPE OF TEST	SUBJECT	TESTED WITH:			
		IODINE, 1:1,000, IN SALINE	IODINE, 1:10,000, IN 0.9% POTASSIUM IODIDE	POTASSIUM IODIDE, 1:100	DIODOTYROSINE 1:200
Intra-cutaneous	J. A. B.	4, pale pink slightly elevated	Negative	Negative	Negative
	Control 1	3, pale pink spot	Negative	Negative	Negative
	Control 2	3, faintly pink faintly elevated	Negative	Negative	Negative

When a vaseline salve containing only 0.25 per cent iodine was applied, no reaction occurred, whereas a 1.0 per cent salve, similarly prepared, caused irritation to normal skin. An attempt was made to increase this limited range of practicable ointment concentrations by the use of a method previously described for poison ivy hypersensitiveness in guinea pigs.¹⁴ In this technique a high concentration of the test substance is applied, being removed after a short time by a suitable agent. To this end a 5.0 per cent ointment of iodine in vaseline, carefully ground and otherwise prepared as above, was applied to the forearm of our patient and washed off after twenty to thirty minutes with soap and water, followed by tenth normal sodium thiosulfate solution. The test was read the next day. Controls were uniformly negative, whereas J. A. B. showed a marked reaction of the type described above (Table III). With the above technique, ointment concentrations up to 10.0 per cent iodine may be used. In this way differences between the sensitized individual and normal controls were made more striking.

In contrast to the markedly positive patch tests to iodine salve, intracutaneous tests on J.A.B (0.02 c.c.) with a 1:1,000 solution of iodine were essentially negative. It should be pointed out, however, that this concentration of iodine is slightly less than the weakest solutions capable of giving a patch test in this individual. An intracutaneous test with 1.0 per cent potassium iodide was likewise negative.

In order to gain further information concerning the mechanism of these reactions, it seemed important to study the response of J. A. B. to iodinated protein, since a positive reaction would indicate that the iodine had combined with protein in the body to form an antigen containing the specific group of iodoproteins, diiodotyrosine. It is known that this reaction can easily take place under these conditions,¹⁵ and reactions to formolized proteins in an individual hypersensitive to formaldehyde have been reported by Horsfall.¹⁶ Accordingly, several preparations of iodinated human serum were made by Wormall's method,¹⁷ and various tests were carried out on this patient and on suitable controls. A typical protocol is given in Table II.

TABLE II
TESTS WITH IODINATED PROTEIN

The readings are after fifteen minutes for the immediate reactions and after twenty-four hours for all others. Dilutions were made in saline and are expressed in terms of dry solids. Human serum was used in all the serum preparations. Measurements are in terms of millimeters.

SUBJECT	INTRACUTANEOUS TESTS WITH IODINATED SERUM				PATCH TESTS WITH:	
	0.05%		1.0%		PRECIPITATED IODINATED SERUM (WET SOLID)	DIIODOTYROSINE POWDER
	IMMEDIATE	DELAYED	IMMEDIATE	DELAYED		
J. A. B.	Wheal 8 Flare 19	8, pale pink, slightly elevated	Wheal 12 Flare 50	45, faintly pink, slightly thickened	Negative	Negative
Control 1	Wheal 9 Flare 20	3, pale pink spot	Wheal 11 Flare 50	30, faintly pink, faintly thickened	Negative	Negative
Control 2	Wheal 8 Flare 20	4, faintly pink	Wheal 7 Flare 40	35, very faint- ly pink, no thickening	Negative	Negative

It will be noted that, as far as immediate reactions are concerned, no marked differences were noted between J. A. B. and the controls. This is interesting because strongly positive reactions would be expected in human hypersensitivity to iodinated protein. Such differences in delayed reactions as occurred may not have been enough to be significant. While these tests were being carried out, it happened that another individual, J. B. H., hypersensitive to formaldehyde, was available. J. B. H. was tested for hypersensitivity to formalized human serum, but, as in the above instance with iodinated proteins, the outcome was essentially negative.

Failure of patch tests with precipitated iodinated protein, and diiodotyrosine, the specific grouping in iodinated proteins, was quite definite. Intracutaneous tests with diiodotyrosine were likewise negative (Table I). No generalized reaction was observed following any of the tests, although local reactions from

the patch tests at times were exceedingly severe. The responses observed were exclusively of the delayed type.

Since hypersensitiveness is a highly selective phenomenon, it was of interest to learn whether idiosyncrasy to iodine is subject to the same law of specificity. To obtain this information cross tests were performed with iodine and formaldehyde on J. A. B. and J. B. II. The method of testing and results noted are given in Table III.

TABLE III
SPECIFICITY OF IDIOSYNCRASY TO IODINE
Readings made on the day following the test

SUBJECT	TESTED WITH:		
	5% IODINE IN VASELINE WASHED OFF AFTER 15 MINUTES WITH SODIUM THIOSULFATE	LANOLIN SALVE CONTAIN- ING 10% FORMALDEHYDE —NOT WASHED OFF	LANOLIN—NOT WASHED OFF
J. A. B.	Pale pink, slight vesiculation	Negative	Negative
J. B. H.	Negative	Pink, thickened, and rough	Negative
Control 1	Negative	Negative	Negative
Control 2	Negative	Negative	Negative

It will be observed that with these two substances, iodine and formaldehyde, the specificity was definite. J. A. B., hypersensitive to iodine, had a pink, slightly vesiculated lesion twenty-four hours after treatment with iodine salve, but gave no trace of reaction with formaldehyde salve. In J. B. II. the opposite reactions were observed. Thus these idiosyncrasies appear to be as specific as other immunologic phenomena. Since hypersensitivity to lanolin has been reported, a control test of this substance was included.

DISCUSSION

Interpretation of these studies is necessarily limited by the fact that they were obtained from a single experimental subject. However, certain points seem to be clear. The condition known as contact sensitivity to iodine is a manifestation of hypersensitiveness and is characterized by strict specificity. All intracutaneous tests with iodine solutions or iodinated protein were negative or equivocal.

In regard to the failure to obtain immediate reactions to intraeutaneous injections of iodinated protein, it should be considered that a single negative result of this sort does not carry much weight. However, the tests were repeated a number of times with different preparations and concentrations, and the opportunity for technical errors would not seem to be great. It would seem that Wolff-Eisner's hypothesis¹⁸ that these idiosyncrasies are due to the formation of an antigen by the combination of iodine with body constituents has not yet been proved. It may be recalled, however, that reactions with protein combinations have been obtained in guinea pigs¹⁹ sensitized to simple chemical substances.

The absence, noted in Table I, of a dermal reaction to a simple alcoholic solution of iodine (no potassium iodide) was observed twice but needs further confirmation since tests were performed with a minimal quantity of material in an attempt to avoid unnecessary reactions in this very hypersensitive patient.

SUMMARY

An individual giving marked contact reactions with iodine was examined. The specificity of the reaction and methods of testing were studied. Intradermal tests were essentially negative. No conclusive evidence of sensitivity to potassium iodide, diiodotyrosine, or iodinated protein was observed.

REFERENCES

1. deBroe, M. Patris: Intoxication Iodique Mortelle, *Arch. de méd. et pharm. mil.* 57: 96, 1911.
2. Herzog, W.: Zur Anwendung der Iodtinktur, *München. med. Wehnschr.* 61: 2319, 1914; The Dangers of Tincture of Iodine as a First-Aid Dressing, *Brit. M. J.* 1: 441, 1915.
3. Bryan, R. C.: One Hundred Prostatectomies, *Virginia M. Monthly* 48: 338, 1921.
4. Jones, O. T.: Dermatitis Consequent on Application of Iodine, *Brit. M. J.* 1: 14, 1931.
5. Rutherford, W. J.: Excessive Reaction Following the Use of Tincture of Iodine, *Brit. M. J.* 1: 846, 1915.
6. Clifford, S. H.: Iodine Hypersensitization, *Boston M. & S. J.* 195: 931, 1926.
7. Brack, W.: Untersuchungen an einem Fall von Iodüberempfindlichkeit, *Dermat. Ztschr.* 56: 26, 1929.
8. Alexander, R. C.: Fatal Dermatitis Following Use of Iodine Spirit Solution, *Brit. M. J.* 2: 100, 1930.
9. Brenas and Weis: Dermite Bulleuse Géante de l'abdomen chez un Enfant, *Bull. Soc. franç. de dermat. et syph.* 40: 19, 1933.
10. Garrido, J. A.: Iodism Following Percutaneous Application of Iodine, *El Siglo. Med.* 97: 338, 1936.
11. Seymour, W. B., Jr.: Poisoning From Cutaneous Application of Iodine, *Arch. Int. Med.* 59: 952, 1937.
12. Rowell, H. G.: Surgical Importance of Iodine Idiosyncrasy and Poisoning, *Surg., Gynec. & Obst.* 36: 219, 1923.
13. Biesalsky: Discussion of a Case, *Deutsche Gesellsch. f. Chir.* 39: 34, 1910; Meszkowicz, *Ibid.*, p. 40.
14. Jacobs, J. L., and Welcker, M. L.: A Method of Testing Guinea Pigs for Hypersensitiveness to *Rhus toxicodendron*, *Proc. Soc. Exper. Biol. & Med.* 38: 726, 1938.
15. Jacobs, J.: Serological Studies on Iodinated Sera, I. Precipitins and Precipitinogens, *J. Immunol.* 23: 361, 1932.
16. Horsfall, F. L., Jr.: Formaldehyde Hypersensitiveness, *J. Immunol.* 27: 569, 1934.
17. Wormall, A.: Immunological Specificity of Chemically Altered Proteins, *J. Exper. Med.* 51: 295, 1930.
18. Wolff-Eisner, A.: Ueber die Urticaria vom Standpunkt der neuen Erfahrungen über Körperfremdem Eiweisssubstanzen, *Dermat. Centralbl.* 10: 164, 1906-07.
19. Landsteiner, K., and Jacobs, J.: Studies on the Sensitization of Animals With Simple Chemical Compounds II., *J. Exper. Med.* 64: 625, 1936.

ACUTE ENDOCARDITIS DUE TO AN ANAEROBIC PNEUMOCOCCUS*

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IN 1936 three strains of anaerobic pneumococci were described by Smith.¹ Of these, two different strains were isolated from the throat of a patient with chronic bronchitis and one was obtained by post-mortem culture from the lung in a patient with pneumonia. White² cites a personal communication from Avery regarding the occurrence of anaerobic pneumococci. We have not been able to find any other descriptions of anaerobic pneumococci. The following case is the first instance in which such organisms have been isolated from the blood stream during life and also the first case of acute endocarditis caused by an anaerobic pneumococcus.

REPORT OF CASE

D. L. (Hospital No. 221450), a 9-year-old boy, was admitted to the Pediatric Service on April 22, 1939, with the following history: Thirteen days prior to admission, he complained of a headache on the left side of his head. Daily fluctuations in temperature from 98.6° to 104° F. were observed. On the third day of his illness, there developed swelling and redness of the left eye, and pain in the neck without rigidity. Eight days before admission, a cardiac murmur was heard for the first time by the family physician. During the three days before admission four chills occurred, with temperature elevations reaching 105.2° F.

Physical Examination: The patient was well nourished, well developed, and acutely ill. There was injection of the nose and pharynx and slight enlargement of the cervical lymph nodes. The heart was not enlarged but there was present a high-pitched musical systolic murmur at the apex, transmitted to the axilla. The spleen was palpable 0.5 cm. below the costal margin and was firm but not tender. The liver was palpable 1.5 cm. below the costal margin. The swelling of the eye was no longer evident and the pains in the neck had disappeared. The laboratory data were as follows: hemoglobin 70 per cent, erythrocytes 3,570,000 per cmm., leucocytes 12,500 per cmm., with 82 per cent polymorphonuclear cells, of which 23 per cent were band forms, 14 per cent lymphocytes, and 4 per cent monocytes. The urine was negative.

Course: At 10:00 p.m. on the day of admission the patient had a chill with a rise of temperature to 105° F. A blood culture taken at this time showed a growth of anaerobic pneumococci after three days (the organisms will be described later). On the following day there developed a slight swelling and tenderness of the left knee and left shoulder and hip.

On April 24, two days after admission, the patient showed signs of meningitis with rigidity of the entire spine and neck. The Kernig sign could not be evaluated because of the pain in the left limb. Lumbar puncture revealed a turbid spinal fluid under slightly increased pressure. A cell count of 1020 per cmm., with 93 per cent polymorphonuclear cells and 7 per cent lymphocytes was obtained. The sugar content was normal. A Gram stain of the fluid showed very rare gram-positive diplococci which failed to grow on culture. Blood culture revealed the same organism previously isolated.

Because of the presence of gram-positive cocci in the spinal fluid, intrathecal sulfanilamide and oral sulfapyridine therapy were instituted. Spinal fluid withdrawn immediately before the first intrathecal injection of sulfanilamide contained 760 cells per cmm., 75 per cent being polymorphonuclear cells and 25 per cent lymphocytes. This specimen showed no organisms in the direct smear and no growth on culture.

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By the next morning the temperature had dropped to below 100° F. The tenderness in the lumbosacral region and left leg and the nuchal rigidity had almost completely disappeared. Roentgenographic examination of the hip and pelvis was negative. There was present definite haziness in the left frontal, ethmoidal, and maxillary sinuses. (Cultures were not obtained directly from the sinuses, but nose and throat cultures showed no anaerobic pneumococci.) During the next week the temperature remained below 100° F. and the child's condition appeared to improve.

On May 2 a tender erythematous area, 3 cm. in diameter, was seen on the dorsum of the right foot. On May 4 and May 6 the patient had chills with a positive and negative blood culture, respectively. On the latter date he complained of severe pain in the left hand which showed tenderness and swelling of the metacarpal region and redness and swelling of its volar surface. In the evening of the same day a purpuric rash appeared on the volar surfaces of both hands and the plantar surface of the right foot. The rash consisted of reddish-violet spots, about 0.5 cm. in diameter, which were not elevated and which faded on pressure. Cultures of these areas were negative. The spleen was palpable 3 cm. below the costal margin and was hard, firm, and not tender. The fundi were normal. By May 14 the rash was almost gone. The patient had a yellowish pallor but no jaundice. There was pain in the right elbow which, however, was not red or swollen. The murmur was much louder and scattered sibilant râles were heard over both lung fields. The liver could now be felt 4 cm. below the costal margin.

On May 16 the face was flushed and had a cyanotic tinge. There was present in each eye a hemorrhage at the margin between the inferior bulbar and palpebral conjunctivae. On May 20 the patient became irrational and delirious. Convulsive seizures occurred, starting on the left side and becoming generalized in a few seconds. During these seizures the right arm and leg were spastic. The course continued steadily downhill and the patient died on May 22. A lumbar puncture shortly before death showed a turbid fluid with innumerable gram-positive diplococci. On culture these organisms proved to be identical with those isolated from the blood. Consent for autopsy was not given.

BACTERIOLOGIC STUDIES

The organism was a small gram-positive lancet-shaped diplococcus which could be grown only under anaerobic conditions. The colonies, like those of pneumococci, were flat and surrounded by an area of greenish discoloration. It was bile soluble but did not give a "Quellung" reaction with the sera of the known types. Fermentation with acid and clot formation occurred in inulin, galactose, lactose, maltose, saccharose, mannite, sorbitol, dextrose, salicin, glycerol, and levulose serum media. The strain isolated by Smith differed in that no reaction took place in mannite, and acid only was formed in sorbitol and glycerol.

Per se the organism was not pathogenic for mice. When injected together with mucin, however, the mice were killed in eighteen hours by a suspension containing 150,000,000 organisms per cubic millimeter and positive cultures were obtained from the peritoneal exudate and heart's blood. In rabbits the intravenous injection of as much as 2 c.mm. of an eighteen-hour broth culture caused a transient bacteremia which did not have a fatal outcome. When the animals were sacrificed six weeks after the injection, the only lesions present were a few small abscesses in the liver. Intraentaneous inoculation of 1 c.mm. produced the same picture except for the presence of a huge local abscess around the site of the injection. Guinea pigs were killed by the intraperitoneal injection of 1 c.mm. of an eighteen-hour broth culture, although 0.1 c.mm. was not fatal.

Mice infected with the organism-mucin mixture were not protected by either sulfapyridine or sulfanilamide. Control experiments with a virulent pneumococcus revealed that mice could not be protected against suspensions of the same density, although the therapy was effective against smaller numbers of lethal doses. Obviously, no conclusion can be drawn concerning the susceptibility of our strain to sulfapyridine.

Rabbits were immunized by repeated intravenous injections of a suspension of killed organisms. The antiserum produced gave positive "Quellung," agglutination, and protection tests with the homologous strain. No "Quellung" reaction was obtained with any of the 32 known serologic strains, and agglutination and protection tests with types I, II, V, VI, and XIV were negative.

For approximately four months after its isolation, the organisms could be grown only under anaerobic conditions. After this period, however, it began to grow aerobically. At first there was a scanty growth but within five days it grew luxuriantly. The aerobic strain was identical with the anaerobic in its fermentation and serologic reactions, its virulence for mice, and its behavior to sulfapyridine.

DISCUSSION

A case of acute endocarditis and meningitis caused by an anaerobic pneumococcus is reported. The onset with headache and swelling of the eye and the roentgenographic evidence of infection in the sinuses indicate that these were the primary foci, although in the absence of positive cultures this cannot be stated definitely.

Since soluble sulfapyridine was not then available, intrathecal sulfanilamide was given and maintained at a level of 7.6 mg. per cent for five days. The effect of this therapy is difficult to evaluate. As seen from Table I, the second spinal fluid was already sterile and the number of cells had fallen from 1,020 to 760 per cubic millimeter. The subsequent fluids, except for the last one, were all sterile and showed a steady decrease in the cell count. The intrathecal therapy was discontinued when the number of cells had fallen to 29.

Concurrently with the intrathecal sulfanilamide, oral sulfapyridine was instituted. On April 26, two days later, a blood level of 15 mg. per cent was reached. During the next week a total of 725 grains of sulfapyridine was given. The temperature in this period was below 100° F. On May 4 the patient suffered a chill with a rise in temperature to 104.2° F. A blood culture at this time was positive. The concentration of sulfapyridine in the blood which had been 9.4 mg. per cent the previous day had fallen to 5.7 mg. per cent. The spinal fluid showed no organisms and had only 21 cells per cubic millimeter. Since the temperature remained high and the blood sulfapyridine could not be raised above 6.9 mg. per cent by oral administration, a soluble sulfapyridine compound (7 per cent solution) was given intravenously. After two intravenous injections the temperature curve appeared to take a downward trend. On the next day, despite a sulfapyridine level of 11.1 mg. per cent, a positive blood culture was obtained. For the next two days the temperature ranged between 99° and 101° F. Following the third injection, however, a rise in temperature occurred and the intravenous therapy was dis-

TABLE I
SPINAL FLUIDS

DATE	PRESSURE	APPEARANCE	SMEAR	CULTURE	CELLS	SULFAPYRIDINE CONCENTRATION (MG. %)
4/24	Slight increase	Turbid	Gram-positive diplococci (rare)	No growth	1,020 (93% polys.) (70% lymph.)	
4/25	Slight increase	Turbid	No organisms	Sterile	760 (75% polys.) (25% lymph.)	5.0
4/26	Slight increase	Turbid	No organisms	Sterile	860 (69% polys.) (31% lymph.)	6.5
4/27	Slight increase	Clear	No organisms	Sterile	220 (79% polys.) (21% lymph.)	7.9
4/28	Normal	Clear	No organisms	Sterile	96 (80% polys.) (20% lymph.)	8.4
4/29	Normal	Clear	No organisms	Sterile	70 (52% polys.) (48% lymph.)	7.8
4/30	Normal	Clear	No organisms	Sterile	29 (62% polys.) (38% lymph.)	8.4
5/ 1	Normal	Clear	No organisms	Sterile	32 (37% polys.) (63% lymph.)	
5/ 4	Slight increase	Clear	No organisms	Sterile	21 (48% polys.) (52% lymph.)	
5/22	Slight increase	Turbid	Innumerable gram-positive diplococci	Anaerobic pneumococcus		

TABLE II
BLOOD

DATE	CULTURE	SULFAPYRIDINE CONCENTRATION (MG. %)
4/22	Anaerobic pneumococcus	
4/24	Anaerobic pneumococcus	
4/26		15.0
5/ 3		9.4
5/ 4	Anaerobic pneumococcus	5.7
5/ 6	Sterile	
5/ 9	Sterile	6.9
5/11	Anaerobic pneumococcus	11.1
5/18	Anaerobic pneumococcus	3.2

continued. The patient continued to receive 60 grains of sulfapyridine daily by mouth. Nevertheless, one week later the blood level was only 3.2 mg. per cent. From Table II it is obvious that there was no relationship between the outcome of the cultures and the concentration of sulfapyridine in the blood. This fact was given added support by our experiment which showed the resistance of the organism to the drug.

Data concerning the frequency of the occurrence of anaerobic pneumococci is not available, since they are, as a rule, not sought for routinely. Our observations suggest that further investigation along these lines is needed.

SUMMARY

1. A case of acute endocarditis and meningitis caused by an anaerobic pneumococcus of a new serologic type is reported.

2. The identity of the organism was established by its solubility in bile, fermentation of inulin, and "Quellung" reaction with an homologous immune serum.

3. These organisms were not pathogenic for mice, which could be killed by the injection of a heavy suspension in mucus.

4. Mice infected with mucus suspensions of these organisms were not protected by either sulfanilamide or sulfapyridine.

We wish to express our gratitude to Mrs. Anita Rosenfeld for her technical assistance.

ADDENDUM: In a recent article Walter and her co-workers¹ described a new type of pneumococcus which they designated as type 33. Miss Walter was kind enough to examine our strain and found it to be of the same type.

REFERENCES

1. Smith, F.: Anaerobic Pneumococcus, Brit. J. Exper. Path. 17: 329, 1936.
2. White, B.: The Biology of Pneumococci, New York, 1936, The Commonwealth Fund, p. 44.
3. Walter, A. W., Blount, K. D., Beattie, M. W., and Cotler, H. Y.: J. Infect. Dis. 66: 181, 1940.

A QUALITATIVE AND QUANTITATIVE STUDY OF ATMOSPHERIC POLLEN AT MOSCOW, IDAHO

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THE collections of atmospheric pollen grains used in this study were obtained by exposing gelatin-coated microscope slides on top of Morrill Hall on the campus of the University of Idaho at Moscow. The campus is on a hill, and the roof of Morrill Hall, a four-story building, is high enough that the local vegetation has a minimum effect upon the composition of the pollen of the atmosphere.

An attempt was made to collect pollen for every twenty-four-hour period. This was not always possible, and as many as six days elapsed between some of the samples.

The slides were prepared according to Wodehouse's¹ glycerin jelly method, and the stain, basic fuchsin, was prepared according to the method of Chamberlain.² The slides were exposed 6 inches below the flat roof of a small especially constructed shelter. After exposure the slides were stored in cream sample bottles to keep them from gathering more pollen and dust before the cover slips were applied. They could be stored in this way indefinitely if rainy weather did not excessively moisten the gelatin so as to permit mold spores to germinate.

Wodehouse's¹ key for the identification of pollen grains was used, and as further aids in identification, observations were made on the phenology of plants in the Moscow vicinity, and reference mounts were prepared from fresh or from herbarium specimens.

Slides were exposed from February 19 until December 2, although no pollen was found on them before March 5 or after November 30.

The first pollen caught was three grains of grass pollen which appeared on March 5 (see Chart 1). Since no other grains of this type were caught until late in April, these grains were apparently produced the previous year. Other pollens appearing too early for the regular seasons were from *Pinus*, *Artemisia*, *Chenopodiaceae*, or *Amaranthaceae*, and of the *Anthemis* and *Rumex* types. Pine sheds its pollen most abundantly in June, but old staminate cones were found shedding pollen in midwinter of 1939 shortly after this study was discontinued. This indicates a possible source of at least some of the stray grains collected out of the normal season. Duke and Durham³ state that rain washes from the pollen grains the active principle causing allergy, so that these few out-of-season grains can be completely ignored in using the data in the treatment of pollinosis.

Pollen Type	Months										Maximum Count	Date of Maximum
	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.			
<i>Pinus</i>											59	Jun. 1
<i>Picea</i>											3	Jun. 13
<i>Abies grandis</i>											19	Jun. 11
<i>Pseudotsuga taxifolia</i>											19	Apr. 25
<i>Tsuga heterophylla</i>											19	May 24
<i>Thuja</i>											77	Apr. 2
Gramineae											214	Jun. 13
<i>Carex</i>											2	Apr. 26
Juncaceae											2	May 17
<i>Salix</i>											60	Jun. 13
<i>Populus</i>											42	Apr. 22
<i>Juglans nigra</i>											6	Jun. 2
<i>Betula</i>											13	Apr. 27
<i>Alnus</i>											13	Jun. 1
<i>Castanea</i>											14	Jun. 2
<i>Ulmus</i>											53	Apr. 5
<i>Celtis</i>											1	Jun. 25
<i>Rumex</i> type											8	Jun. 1
<i>Eriogonum</i> type											3	Apr. 25
Chenopodiaceae-Amaranthaceae											62	Apr. 15
<i>Platanus</i>											1	Jul. 4
<i>Acer saccharinum</i>											30	Apr. 2
<i>Acer negundo</i>											24	Apr. 25
<i>Acer</i> (other species)											6	May 24
<i>Cornus stolonifera</i>											1	Jun. 12
<i>Fraxinus</i>											13	May 10
Oleaceae (not <i>Fraxinus</i>)											1	
<i>Plantago</i>											5	Jul. 7
Cichorieae											4	Aug. 13
Astereae											1	May 17
<i>Heliantheae</i>											1	
<i>Anthemis</i> type											20	Jul. 11
<i>Artemisia</i>											150	Sep. 24
<i>Iva</i>											4	Jun. 3
<i>Ambrosia-Franseria</i>											13	Sep. 15

Chart 1.—Pollen types collected at Moscow during 1938. Shaded area indicates the total span of the pollination season; black area indicates the interval between the first and last days on which five or more grains fell on the sample area of gelatin (3.24 cm.).

Although pollen analysts in other regions have distinguished between species belonging to the *Chenopodiaceae* and *Amaranthaceae*, this distinction was found impossible at Moscow. Table I, showing the measurements of a series of grains collected in early summer, indicates the difficulty encountered.

The local flora includes about twenty species in the *Chenopodiaceae* (divided among *Atriplex*, *Chenopodium*, and *Salsola*) and four species of *Amaranthus*. The data in Table I show that the measurements of the pollen grains of this particular flora overlap to an extent that the distinction of the species is practically impossible.

TABLE I

NO. OF GRAINS	DIAMETER OF GRAIN	DIAMETER OF PORE	DISTANCE BETWEEN PORES
	mμ	mμ	mμ
1	21.58	1.66	4.15
1	23.24	2.49	5.81
1	23.24	3.32	5.81
1	24.90	1.66	2.49
1	24.90	1.66	4.15
1	24.90	2.49	9.96
1	24.90	2.49	3.32
1	24.90	2.49	4.40
2	24.90	2.49	4.15
1	24.90	2.49	6.64
2	24.90	3.32	6.64
1	24.90	3.32	4.98
1	24.90	3.32	8.30
1	26.56	2.49	6.64
1	26.56	3.32	6.64
2	26.56	3.32	4.98
1	26.56	4.15	5.81
1	28.22	3.32	4.98
1	28.22	3.32	8.30
1	29.88	1.66	2.49
1	29.88	3.32	4.98
1	29.88	2.49	4.98

Four per cent of the total grains collected during the year were received in a crushed condition, or were of unrecognized types.

Examination of weather records,⁴ particularly the deviations from normal precipitation and temperature, shows that during the months when this study was made, the weather was slightly drier and warmer than the forty-six-year averages. According to Durham,⁵ the dates of onset, crises, and close of the pollen seasons are practically the same at a given locality each year, except in cases of extreme drought. The weather of 1938 so closely approximated the normal climate that the pollen data presented in Chart 1 are probably typical of average years.

I wish to thank Dr. R. F. Daubenmire for suggesting the problem and directing the research, and Dr. A. E. Brann and Mr. Arthur Nelson for assistance in exposing slides during my absence from Moscow.

REFERENCES

1. Wodehouse, R. P.: Pollen Grains: Their Structure, Identification and Significance in Science and Medicine, New York, 1935, McGraw-Hill Book Co., p. 574.
2. Chamberlain, C. J.: Methods in Plant Histology, ed. 5, Chicago, 1932, Univ. of Chicago Press, p. 405.
3. Duke, W. W., and Durham, O. C.: Pollen Content of the Air, J. A. M. A. 90: 1529, 1928.
4. United States Department of Agriculture Weather Bureau: Climatological Data, Idaho Section, Annual Summary 41 (13): 57-62, 1938.
5. Durham, O. C.: The Pollen Content of the Air in North America, J. Allergy 6: 128, 1935.

INTRAMUSCULAR ADMINISTRATION OF SODIUM SULFAPYRIDINE*

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INTRAVENOUS and oral administrations of sodium sulfapyridine have been up to now the only routes used in the United States. Most American clinicians have administered the drug intravenously,¹ and many reports have appeared in the literature on this means of administration.^{2, 3, 5, 6} However, the intramuscular route offers certain advantages over the intravenous route if the drug can be given with equal safety. Gaisford⁴ and others have preferred to give the drug intramuscularly.

Before recommending this type of treatment, one should consider certain experimental work. Recent reports of experimental intramuscular injections in guinea pigs showed such therapy to be dangerous and impractical, at least in the guinea pig. Necrotic sloughs occurred frequently, and local reactions were many.⁵ In human muscles, however, the mass of muscle is larger and the total quantity of extracellular space that apparently contains large quantities of chloride is greater, thus providing a factor of safety. Those clinicians opposed to the intramuscular route of administration also contend that this method is likely to produce local irritation and that, even if the drug is deposited into the muscle, a depot may be formed from which prolonged absorption can take place. Furthermore, because of the high pH (11.5) of the material used as compared with normal body fluids, it is argued that this solution is far too alkaline and caustic to be employed with safety. We have not found this to be so.

During the past five months 25 patients have been treated with more than 350 intramuscular injections of the drug.

METHOD OF ADMINISTRATION

A 33 $\frac{1}{3}$ per cent solution of sodium sulfapyridine in sterile water was prepared freshly with each dose of the drug. This preparation was injected deeply into the gluteal or thigh muscles, care being taken that none of the material was deposited into the subcutaneous tissue through the needle tract either upon injection or upon withdrawal of the needle. The schedule of dosage followed closely that used in the oral administration of the drug. Each cubic centimeter of the solution represents 0.33 Gm. At the first injection from 6 to 10 c.c. were given, followed by 3 c.c. every four hours, until the temperature was normal for twenty-four hours. Thereafter the dose was reduced one-half, and its administration was continued for the next five or six days.

*From the Department of Medicine, College of Medicine, University of Nebraska. The material used in this study was furnished by Merck & Co. Received for publication, June 27, 1940.

DISEASES STUDIED

Treatment was not limited to cases of pneumococcal infections but included several other diseases, with varying degrees of clinical success. They are included in this series to demonstrate the efficacy of the route of administration rather than to stress clinical effects of the drug.

ANALYSIS OF DATA

Table I shows a record of treatment of 25 persons with infectious diseases, 14 of whom had pneumonia. Diagnoses were confirmed by culture, typing, and x-ray studies whenever possible. Concentration studies of the drug in the blood were likewise made, and these are recorded. The blood levels recorded in the table represent figures obtained after or during intramuscular injections and before oral administration had been started. These blood levels closely parallel those obtained with the same amount of the drug administered orally. It will be noted that the temperature responses are almost identical with those following the oral administration of the drug. The clinical results appeared to be as good as those obtained by other methods of administration. Toxic effects were strikingly few or extremely mild when noted. In the 25 patients studied there were no local effects of irritation or tumefaction. The patients reported no more pain attendant upon the intramuscular procedure than has been noted after any other parenteral therapy in common use. The only toxic effects noted were four instances of nausea and vomiting; three of these patients had been vomiting before the drug was given. No instances of agranulocytosis, massive red blood cell destruction, or skin reactions were noted in the series.

DISCUSSION

It is not the purpose of this communication to urge the intramuscular administration of sodium sulfapyridine to the exclusion of other routes. However, if it can be shown that this method is a safe procedure, nearly everyone will acknowledge that the technical advantages of this route of administration are many.

Some investigators have noted that muscles are capable of storing metallic drugs as well as other materials with no apparent damage and that absorption takes place thereafter. If such theoretical depots or storage reservoirs are produced, they do not appear to cause demonstrable effects. It is also of interest that adequate levels (3 to 10 mg. per cent) of the drug in the blood may be obtained promptly by this method. Moreover—an important point—once the level is reached, it can be maintained for much more protracted periods than is the case with intravenous therapy. Fenn and his associates⁷ in 1934 and Sandow⁸ in December, 1939, pointed out that 15 per cent of muscle volume is extracellular space, in which all the chloride is believed to reside. If this is the case, it may furnish an explanation for the ease with which a muscle is able to handle the very alkaline sodium sulfapyridine solution.

The technical advantages of giving any drug intramuscularly are many if the procedure is otherwise safe. Gastrointestinal reactions to the drug are avoided. Nausea is usually a very constant symptom when the drug is given

TABLE I
SUMMARY OF DATA CONCERNING INTRAMUSCULAR USE OF SODIUM SULFAPYRIDINE

NO.	PATIENT	DIAGNOSIS	BACTERIOLOGY	NO. OF INJECTIONS	TOTAL AMOUNT OF DRUG (GAL.)	BLOOD LEVEL (MG. %)	TEMPERATURE RESPONSE (F.°)	TOXIC EFFECTS	OUTCOME
1	H. B.	Lobar pneumonia	Positive blood culture Type III	32 in 11 days, none orally	Im.—34	3 to 5	104° to normal in 48 hr.	None	Well
2	A. B.	Lobar pneumonia	Type III	26 in 6 days, none orally	Im.—38	2.3 to 5.4	103° to 100° in 48 hr.	None	Well
3	A. M.	Lobar pneumonia	Type III	24 in 7 days, none orally	Im.—27	3.3 to 5.4	102° to normal in 48 hr.	None	Well
4	H. B.	Lobar pneumonia, pregnancy	Type I	41 in 12 days, none orally	Im.—44	4.4 (10 hours)	104° to normal in 54 hr.	None	Improved
5	J. M.	Lobar pneumonia	Type I	11 in 3 days, then orally	Im.—11 O—4		103.8° to normal in 24 hr.	Vomiting began before drug was given	Well
6	E. B.	Lobar pneumonia	Type II	1 dose intramuscularly, then orally	Im.—2.3 O—14		102° to normal in 3rd day	None	Well
7	R. E.	Lobar pneumonia	Type I	2 doses intramuscularly, then orally	Im.—4 O—19		103° to normal in 36 hr.	None	Well
8	J. K.	Lobar pneumonia	Type III	1 dose intramuscularly, then orally	Im.—2.3 O—41		104° to normal in 24 hr.	None	Well
9	W. C. B.	Lobar pneumonia	Type I	2 doses intramuscularly, then orally	Im.—4 O—23		104° to normal in 54 hr.	None	Well
10	W. R. B.	Broncho-pneumonia	Type VI	1 dose intramuscularly, then orally	Im.—2.3 O—12		104° to normal in 18 hr.	None	Well
11	S. V.	Broncho-pneumonia	Type II	1 dose intramuscularly, then orally	Im.—2.3 O—12		102° to normal in 30 hr.	None	Well
12	I. L.	Acute appendicitis, postoperative pneumonia	Type XVII	1 dose intramuscularly, then orally	Im.—2.3 O—8		102° to normal in 12 hr.	None	Well
13	T. L.	Fractured rib, broncho-pneumonia	Diplococci not typed	1 dose intramuscularly, then orally	Im.—2.3 O—29		101° to normal in 3 days	None	Well

14	W. S.	Pneumonia	Streptococcus	1 dose intramuscularly, then orally	Im.—2.3 O—11		102° to normal in 24 hr.	None	Well
15	L. C.	Peritonitis (pneumococcal)		23 in 8 days, then orally	Im.—19.7 O—16	As high as 21.7	104° to normal in 6 days	None	Well
16	W. H.	Undulant fever	Positive agglutination	25 in 5 days, none orally	Im.—27	3.5 in 10 hr.	No definite response	Vomiting began before drug administration was begun	Still in hospital
17	H. F.	Hemiplegia, broncho-pneumonia		36 in 10 days, none orally	Im.—46	2.2 to 2.7	None	None	Died
18	M. R.	Coronary thrombosis, pulmonary infarction		9 in 36 hr., none orally	Im.—10		Questionable	None	Improved
19	F. S.	Appendicitis with perforation		17 in 6 days, then orally	Im.—17 O—27	Less than 3	103° to normal in 3 days	None	Improved
20	E. P.	Appendicitis with peritonitis		5 in 2 days, none orally	Im.—6	6.4 after 24 hr.	None	None	Died
21	R. K.	Erysipela; history of pneumonia preceding	No growth	25 in 10 days, then orally	Im.—29 O—4	4.8	None	None	Died
22	E. L.	Paninusitis, glomerulonephritis		9 in 48 hr., none orally	Im.—9		102° to normal in 12 hr.	Moderate nausea and vomiting	Improved
23	B. T.	Aldison's disease, pharyngitis		26 in 8 days, none orally	Im.—27	8.3	103° to 99°-100° in 24 hr.	Little	Improved
24	P. B.	Diabetes, abscess of leg	Nonhemolytic streptococcus	21 in 7 days, none orally	Im.—21	5.9	None	None	Died
25	D. S.	Typhoid fever	Positive Widal	23 in 8 days, none orally	Im.—25		Questionable	None	Well

by mouth, and the condition even progresses to vomiting, which precludes the administration of the drug. In the treatment of comatose patients it is obviously unwise to attempt to make them swallow drugs or to introduce the drugs into the stomach by tube, since in either instance the aspiration of the drug is likely. Old persons with tortuous and sclerotic vessels, infants, children, and women with inconspicuous or no ample sized veins are usually poor subjects for intravenous therapy. Likewise a safe alternative route of administration is always desirable in those cases in which any factor has made the oral or intravenous routes impracticable or impossible.

CLINICAL RECORD OF A TYPICAL CASE

Mrs. A. M., aged 65 years, was admitted to the University Hospital on Dec. 8, 1939, complaining of chills, fever, and cough with bloody sputum. Seventy-two hours before admission she had been suddenly seized with a chill which was soon followed by fever. She began to cough and shortly thereafter felt a sharp pain in the right chest which was aggravated by deep breathing and by coughing.

Her past history is irrelevant except for two attacks of lobar pneumonia, one in 1931, the other in 1934. Each time she made an uncomplicated recovery.

On admission her temperature was 101° F. and rose to 102° F. within two hours. There was limitation of motion of the right chest, with dullness and decreased breath sounds over its lower half. A few moist râles were heard over the same area. Leucocyte count was 15,200, of which 80 per cent were polymorphonuclear in type. Urine was negative. A roentgenogram of the chest, taken by the portable unit, revealed a diffuse density involving the middle third of the right lung field. Sputum examination revealed type III pneumococci.

At eleven o'clock on the night of admission 6 c.c. of $33\frac{1}{3}$ per cent solution of sodium sulfapyridine were administered intramuscularly, followed in four hours by another 6 c.c. The following morning administration of 3 c.c. of solution every six hours was begun and continued for six days. On the morning after admission, after a total of 4 gm. of the drug had been received intramuscularly, examination showed a blood concentration of 3.3 mg. per 100 c.c. Two days later the blood level was 5.4 mg. per 100 c.c. and still later 4.1 mg. per cent.

The patient's temperature on the second day of admission ranged around 100.5° F.; the following morning it dropped to normal where it remained throughout the remainder of her hospital stay. The chest findings rapidly resolved and disappeared entirely by the seventh day after admission. After the second day the patient felt much better subjectively, and at no time was there any indication of local irritation at the site of injection. The patient was dismissed in good health on Dec. 17, 1939, her tenth hospital day.

CONCLUSIONS

1. Twenty-five cases have been analyzed, in which more than 350 intramuscular injections of $33\frac{1}{3}$ per cent solution of sodium sulfapyridine were given.
2. Clinical results of the drug as manifested by blood levels and temperature response were parallel to those obtained by orally administered sulfapyridine.
3. Extreme care must be exercised to insure the deposition of all the drug into the muscle.
4. Toxic effects were minimal. Local evidences of irritation were not seen.
5. There are many technical advantages to this route of administration.

REFERENCES

1. Marshall, E. K., and Long, P. H.: The Intravenous Use of Sodium Sulfapyridine, *J. A. M. A.* 112: 1672, 1939.
2. Finland, M., Lowell, F. C., Spring, W. C., Jr., and Taylor, F. H. L.: Parenteral Sulfapyridine; The Intravenous Use of Sodium Sulfapyridine and a Report of Clinical and Laboratory Observations on the Use of Glucose-Sulfapyridine Solution, *Ann. Int. Med.* 13: 1105, 1940.
3. Abernethy, T. J., Dowling, H. F., and Hartman, C. R.: The Treatment of Lobar Pneumonia With Sulfapyridine and Sodium Sulfapyridine With Observations Upon Effective Blood Levels, *Ann. Int. Med.* 13: 1121, 1940.
4. Gaisford, W. F., Evans, G. M., and Whitelaw, W.: Parenteral Therapy With M. and B. 693, *Lancet* 2: 69, 1939.
5. Wilson, G. S., and Major, Irene: Treatment With Sulphapyridine (M. and B. 693) of Guinea Pigs Infected With *Brucella abortus*, *Brit. M. J.*, Jan. 13, pp. 47-50, 1940.
6. Sandow, A.: A Geometrical Determination of the Extracellular Space in Muscle, *Proc. Soc. Exper. Biol. & Med.* 42: 772, 773, 1939.
7. Fenn, W. O., Cobb, D. M., and Marsh, B. S.: Sodium and Chloride in Frog Muscle, *Am. J. Physiol.* 110: 261, 1934.

GIANT CELL TUMOR OF THE FRONTAL BONE

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THE case of giant cell tumor of the frontal bone in a boy 13 years old is presented here because of the rarity of the lesion in bones which are of the membranous type. Review of the literature discloses only one previous case which was reported by Fraser¹ in 1931. This patient, aged 42 years, following trauma over the left forehead region, developed a mass. The mass was removed surgically and was found to be a soft tissue tumor which contained giant cells on section. Geschickter and Copeland² reviewed a series of 22 cases of giant cell tumor of the skull bones found at Johns Hopkins Hospital and found no instance of tumor which arose in membranous bone. All these cases could be related to centers of ossification arising in cartilage. They reached the conclusion that benign giant cell tumor could not occur in bones which arise membranously. Cotton³ likewise observed that giant cell tumors do not occur in the bones of the skull.

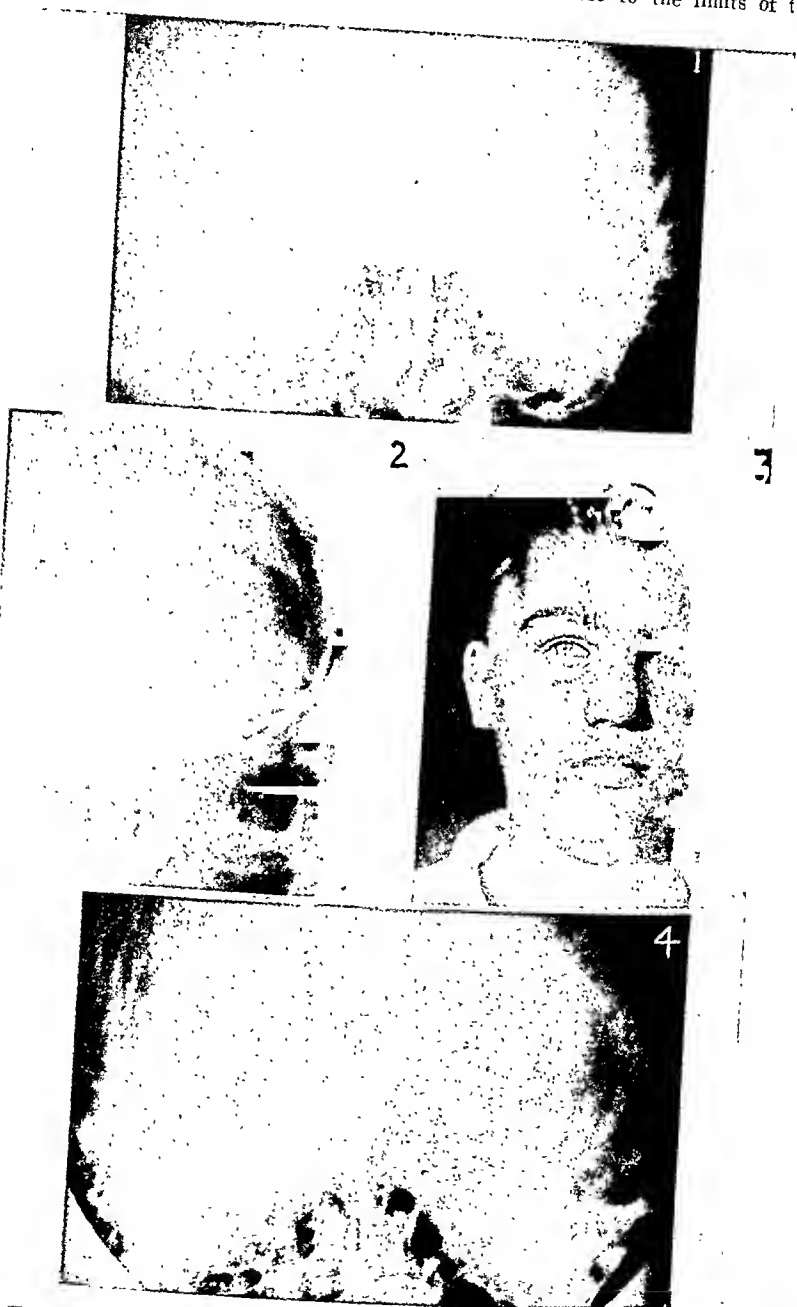
CASE REPORT

A white boy, aged 13 years, was first seen on June 21, 1937, with a protruding mass in the right forehead region. This mass was first noted in 1935, at which time it was thought to be a swelling from an unrecognized trauma. However, progressive enlargement of the tumor mass, which was firm in consistency, painless, and not tender on palpation, discredited this impression. The mass gradually encroached on the right eye, displaced the eye, and restricted its movements. No history of trauma in the region of the tumor mass could be elicited.

The boy was healthy except for the tumor mass above the right eye. In this region there was a smooth, firm, right frontal tumor which measured 5 cm. in diameter and was elevated 1 cm. above the level of the surrounding external table of the frontal bone. The tumor was situated directly above the superior orbital ridge and external angular process of the frontal bone. Laterally it extended across the temporal ridge into the temporal fossa. The right eye was slightly exophthalmic and was displaced medially and downward, with restricted movements laterally and upward.

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Roentgenograms (Figs. 1 and 2) of the skull showed an extensive area of decalcification at the site of the tumor between the thinned inner and outer tables of the frontal bone. Medially a very thin, rounded plate of bone was thought to separate the tumor from a small frontal sinus. The frontal sinuses were clear and symmetrical except for slightly less lateral extension on the right. Laterally the tumor extended almost to the limits of the frontal



- Fig. 1.—Frontal roentgenogram of skull showing encroachment of tumor on right frontal sinus and superior orbital plate.
 Fig. 2.—Right lateral roentgenogram of skull showing extension of tumor into temporal fossa.
 Fig. 3.—Photograph two years postoperative showing site of defect and normal right eye.
 Fig. 4.—Roentgenogram two years and four months postoperative showing filling of bone defect.

bone at the articulation with the sphenoid and malar bones. The inner surface of the tumor was indefinitely outlined by a rounded, partly absorbed, inner table which seemed to protrude into the cranial cavity about 4 cm. The superior orbital plate was well defined but was depressed about 5 mm. from above and laterally. Some evidence of trabeculation could be seen within the tumor although its general density was little greater than the normal frontal sinus. A preoperative diagnosis of right frontal mucocoele was made.

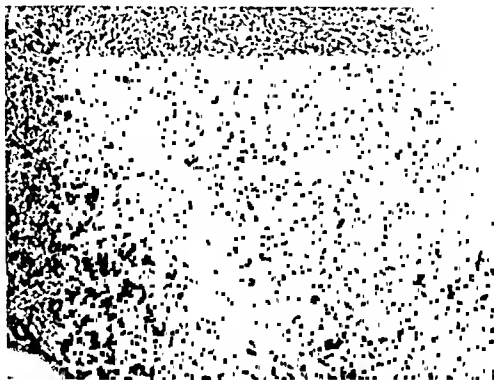


Fig. 5.—Low-power microphotograph of tumor showing numerous giant cells and fibrous stroma.

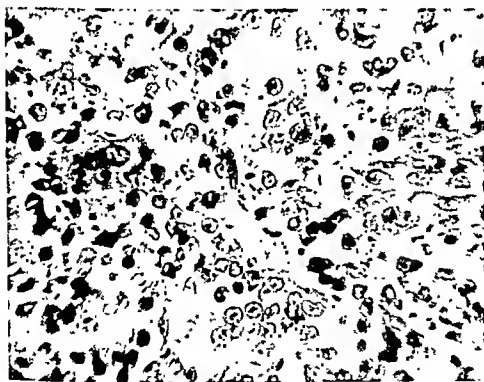


Fig. 6.—High-power microphotograph of tumor showing distribution of nuclei in giant cells.

At operation on July 19, 1937 an opening, 2 by 3 cm., was made through a somewhat roughened and almost perforated thin external table over the tumor. Moderately firm, grayish red tumor tissue was found filling the bone defect. An immediate pathologic report was giant cell tumor, possibly xanthoma. The tumor tissue was removed by blunt dissection and curettage with considerable bleeding. The inner table, which was found to be thin and eroded in places, was partly removed from the dura to reduce its impression on the frontal lobe of the brain and to permit some expansion of the frontal lobe into the large cavity where the tumor had been. The superior orbital plate was not removed where it had been depressed by

the tumor. Hemostasis was obtained with difficulty in the region of the lateral tumor bed by means of electrocoagulation. In this region and at other places in the wall some tumor tissue probably remained. The tumor cavity was filled with normal saline, and the scalp was closed without drainage.

Recovery occurred without aspiration or infection. Roentgen radiation therapy was given over the tumor area from July 26 to July 31, 1937, in a dosage of 1035 r. divided into six daily doses. The blood cholesterol determination on July 26, 1937, was found to be 150 mg. per cent, and the blood calcium was 8.8 mg. per cent, both within normal limits. Fluoroscopic examination of all the bones of the body disclosed no evidence of further bone lesions.

The boy has remained well for more than two years since the operation, and the area operated upon shows very little remaining deformity. The right eye does not appear exophthalmic, and its movements are normal (Fig. 3). Roentgenograms taken on Oct. 27, 1939 (Fig. 4) show the area of bone removed by operation surrounded by a smooth wall, considerably reduced in size with no evidence of recurrence of the tumor.

The tissue removed by the surgeon consisted of 32 Gm. of grayish red hemorrhagic tumor. The tissue was very friable and crumbled easily, except that removed from the periphery which was more firm and had more the appearance of fibrous tissue. These firmer areas extended into the central portion of the tumor.

On section the tumor was found to contain great numbers of multinucleated giant cells. In some areas there were from 20 to 40 giant cells in a low-power field (Fig. 5). The giant cells contained from 5 to 25 oval uniformly staining nuclei which had a tendency to be grouped in the center of the cells (Fig. 6). The cytoplasm of the giant cells was homogeneous and stained well with eosin. The giant cells were more abundant at the periphery of the tumor but were present to a lesser extent throughout the tumor. The stroma of the tumor was very cellular and consisted of round and spindle-shaped cells, the former being most numerous. The nuclei of the round cells were similar in appearance to the nuclei of the giant cells. The cytoplasm of the round cells was small in amount. The tumor contained many blood cells both inside and outside of blood vessels.

CONCLUSION

A case of benign giant cell tumor of the frontal bone has been presented. The location of this tumor in membranous bone is unusual since there is only one other case reported in the literature. The theory of Geschickter and Copeland for the origin of giant cell tumor from resorptive processes in cartilaginous bone seems quite reasonable but does not explain the origin of this tumor. We have been unable to find any evidence in the literature of embryology that cartilage is ever present in the frontal bone. We have made no study of the origin of giant cell tumor and have no theory as to the origin of this tumor.

REFERENCES

1. Fraser, J.: Benign Giant Cell Tumor of Bone, *Clin. J.* 60: 20, 1931.
2. Geschickter, C. F., and Copeland, M. M.: Tumors of Bone, ed. 2, New York, 1936, *Am. J. Cancer*, chap. 12, p. 309.
3. Cotton, F. J.: Notes on Giant Cell Tumor of Bone and Cysts, *New England J. Med.* 210: 1095, 1934.

CLINICAL CHEMISTRY

A THEORY OF PORPHYRINOGENESIS*

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THERE is no one today who can say how hemoglobin is formed or from what sources its pigmented fraction, protoporphyrin, arises. After a century of study of hemoglobin metabolism there is still nothing known of its anabolic aspects. Between the absorption of food and the appearance of porphyrins and porphyrin compounds in the cells of the body and in excreta there lies an unexplored and undoubtedly important gap.

In the absence of any definite information as to how the various porphyrins arise, a number of hypotheses have been broached, all of which fail to satisfy the requirements of heuristic concepts in two major essentials. In the first place, they leave unexplained one or more of the major factors in the problem; in the second place, they offer no points for direct experimental attacks. Any theory of porphyrinogenesis must explain the following facts:

I. The occurrence of the following porphyrins: protoporphyrin 2, protoporphyrin 9, coproporphyrin I, coproporphyrin III, uroporphyrin I, and uroporphyrin III (Fig. I).

II. The normally great predominance of protoporphyrin 9.

III. The normal appearance of small and equal amounts of the two coproporphyrin isomers.

IV. The extremely rare occurrence of the uroporphyrin isomers.

As a matter of historical interest it is worthy of note that Günther,¹ in his 1911 monograph on porphyria, discussed the possibility that there might be a faulty catabolism of hemoglobin, with the production of "hematoporphyrin" in the liver. This view was shortly proved untenable, at least in its original form, by Fischer's² demonstration that the porphyrins excreted in porphyria are coproporphyrin and uroporphyrin, and that in any event they are of an entirely different isomeric type than is the blood porphyrin. Further studies on porphyria have also revealed that the enlarged liver of true congenital porphyria is not functionally impaired either as an excretory or as a metabolic organ,³ and that acute porphyria not infrequently occurs without evidence of liver damage.^{4, 5} It should not now require proof that the "urobilinogen" of the acute porphyric urine is not urobilinogen at all.^{4, 6}

Following his demonstration that the feces of Petry contained the tetracarboxylic acid coproporphyrin, whereas the urine contained predominantly the octacarboxylic acid uroporphyrin, Fischer^{2c} suggested that the former might arise by carboxylation of the latter in the kidney. This has been aban-

*From the Veterans Administration Facility, North Little Rock.

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done in view of the improbability that such a reaction could occur biologically. Further, it was soon found that uroporphyrin existed in Petry bone and in his ascitic fluid.⁷

By 1929, when Borst and Königsdorfer⁸ published their monumental treatise on the pathologic examination of Petry, the München school was in agreement that the synthesis of the three main types of porphyrins ran independent courses. In particular, porphyria was regarded as an error of porphyrin anabolism.

Repeatedly suggested, even today, is the possibility that protoporphyrin may give rise to coproporphyrin. This is based on the old idea that the porphyrin excreted in porphyria is hematoporphyrin. It is to be said here that hematoporphyrin is an entirely artificial product of the chemical laboratory and does not occur in nature. The belief was a part of the theory that there might be a faulty breakdown of hemoglobin in the liver. In 1932 van den Bergh, Grotepass, and Revers⁹ claimed that on perfusion of liver with a solution containing protoporphyrin the bile excreted contained coproporphyrin. This experiment has been widely quoted or tacitly used to support later proponents of a catabolic theory of coproporphyrin formation. Aside from the fact that it has never been repeated, and that Fischer has been unable to corroborate it,¹⁰ the evidence presented by the authors is of the slightest sort. Fischer and Orth¹⁰ say: "Möglicherweise handelt es sich hierbei aber um die leichte verständliche Bildung von Hämatoporphyrin IX, an Stelle des Koproporphyrin III." The objection must also be made that the catabolic theory entirely begs the question as to the origin of protoporphyrin.

Later Schreus¹¹ and Carrié¹² proposed a classification of diseases of porphyrin metabolism based on the type of porphyrin excreted. If type I porphyrins were formed, it would be considered a "primary" fault; if type III, it would be "secondary" to a faulty hemoglobin destruction. While some evidence has been presented for this view, and Vannotti,¹³ in particular, leans to it, recent studies on porphyria have shown such a classification to be wholly untenable. Among the objections to it may be mentioned only the necessity of explaining how protoporphyrin may give rise to coproporphyrin; the failure to explain uroporphyrin or protoporphyrin formation; the failure to explain Dobriner's¹⁴ evidence that coproporphyrin formation is associated with erythropoiesis rather than hemolysis; and the inability to explain the simultaneous excretion of both isomeric types of porphyrins, which is the rule rather than the exception.

The faulty hypotheses of the past have been inadequate in large part because of the lack of sufficient data on which to build soundly. Since the discovery of a porphyria with excretion of coproporphyrin III by van den Bergh, Regniers, and Müller¹⁵ in 1928, there has been a realization that sooner or later isomers of the other known porphyrins would be found to occur in nature. This was demonstrated for the uroporphyrins in 1935 by Waldenström,¹⁶ and in the following year by Mertens¹⁷ and by Fischer and Libowitzky.¹⁸ It has since been abundantly confirmed in human,^{18, 19} bovine,²⁰ and squirrel²¹ porphyria that both isomers are excreted simultaneously. Recently, Mertens²² has re-

vealed indeed that in Petry fees the presence of coproporphyrin III in addition to coproporphyrin I had been overlooked, despite its presence in amounts as great as that of the more easily crystallized coproporphyrin I. This is of the greater importance since Grotelpass²³ has reported that the two coproporphyrin isomers occur in equal amounts in normal urine. Recently, Fischer²⁴ has presented evidence that there is also a dualism of the blood pigments, in that, in addition to the previously identified protoporphyrin 9 obtainable from hemoglobin, there is also a small amount of protoporphyrin 2.

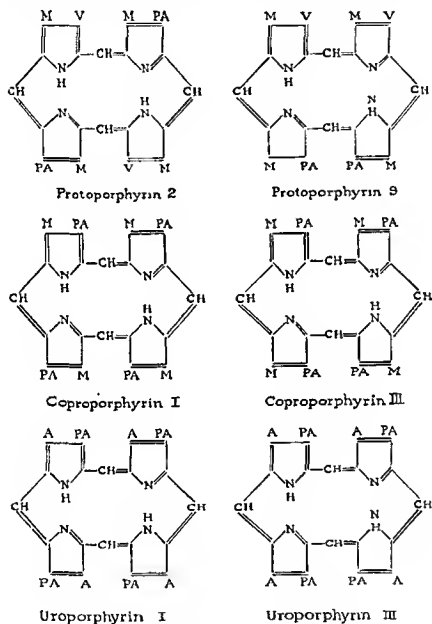


Figure 1

In 1936 Rimington,²⁵ appreciating the dualism of the porphyrins, proposed a schematic theory of enzyme action controlling not only the rate and type of porphyrins production, but also the formation of different porphyrins. His postulates, however, were too generalized for closer definition and merit no attention. In the same year, at the Baltimore meeting of the Southern Medical Society, Dobriner put forth a theory, published in 1937,^{14a} that definitely proposed a chemical mechanism of porphyrinogenesis in keeping with known facts.

According to Dobriner, it is postulated that there are formed two pyrromethenes, A and B, which may combine in any of three possible ways: A + A

will give a type I porphyrin; B + B, a type II porphyrin; A + B, a type III porphyrin.

Type II porphyrins have not so far been discovered in biologic material, and Dobriner was led to suppose an enzymatic action which would act in such manner as to agree with actual facts. It will be noted that this ingenious theory still fails to explain the occurrence of three kinds of porphyrins.

By a glance at the formulas in Fig. I it will be apparent that these three kinds of porphyrins may be considered to be comprised of no more than three structural units; namely, 2-acetic acid, 3-propionic acid pyrrol; 2-methyl, 3-propionic acid pyrrol, and 2-methyl, 3-vinyl pyrrol, of the structures in Fig. III.

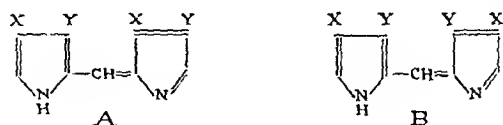


Figure II

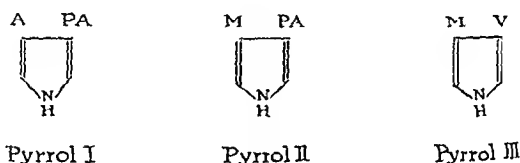


Figure III

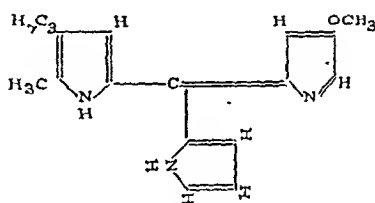


Figure IV

By simple decarboxylation of the acetic acid group pyrrol I is transformed into pyrrol II; dehydrogenation and decarboxylation of pyrrol II leads to pyrrol III. These reactions are well known in biologic processes, and it is not unimaginable that pyrrol I may actually be the primary building block of the naturally occurring porphyrins.

Such a pyrrol may well undergo an aldehyde synthesis to form a pyrromethene. Corwin and Andrews²⁶ have shown that in the aldehyde synthesis of pyrromethenes there is an intermediary formation of a tripyrrylmethane. It is of considerable interest that at least once in the experiments of Nature such a compound has been formed as a blind alley. This compound, prodigiosin, has been given the structure in Fig. IV.²⁷

It may now be seen that if such a tripyrrylmethane were to be formed of the pyrrol I units, it may have the following form, which, on passing to the pyrromethenes, may lead to uroporphyrins I, II, and III:

Such a synthesis must be very rare, for it is seldom found, and it must occur only when the acetic acid group is protected from decarboxylation. Fischer

and Hofmann,²⁸ and Fischer and Müller²⁹ have shown that pyrroles of the type under discussion are unstable, losing carbon dioxide spontaneously at room temperatures.

The formation of an homologous trimethyl, tripropionic acid tripyrryl-methane might explain the formation of the coproporphyrins.

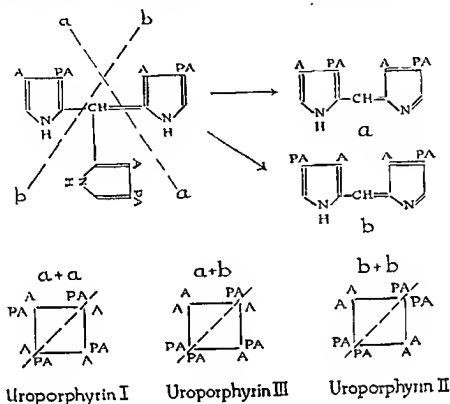


Figure V

However, the predominance of protoporphyrin as the apex of pyrrol metabolism indicates strongly that the extent of formation of pyrrol III must be large. The formation of protoporphyrin 2 and protoporphyrin 9 may then be accounted for by the intermediate formation and decomposition of a tripyrryl-methane according to the course indicated in Fig. VI.

In this theory, as well as in Dobriner's, there is expected the formation of some type II porphyrin (protoporphyrin 5). If the theory is correct, this will be found, unless there is some purely chemical reason, possibly associated with substituents on the 1 or 4 positions of the pyrrole rings, which would preclude it. It is, of course, likely that porphyrins are not formed without the action of enzymes, but of them nothing is known at present. However, the fact that uro- and coproporphyrins almost universally occur as their zinc complexes^{6, 21, 30, 31} suggests that this metal may play a part in their formation.

Concerning the source of the proposed pyrrol I there is little to be said. Certainly it cannot arise from chlorophyll or any of its derivatives. Here it may be pointed out that there is no reason to believe that chlorophyll plays any part in the formation of animal porphyrins. Among the amino acids only tryptophan could conceivably be a precursor of pyrrol I, and this would require the improbable but possible opening of the 6-membered ring. This might occur by an oxidative scheme, such as is known with tyrosine and with quinone (Fig. VII).

However, as has been pointed out by Thomas,³² there is no good evidence that tryptophan is essential in the formation of hemoglobin. Indeed, the work

of Fischer and his students, investigating the production of coproporphyrin by yeast,³³ and the study of Thomas³² with the Harder's glands of rats as an indicator of protoporphyrin production, point to the probability that carbohydrate and simple amine metabolism is closely related to porphyrinogenesis.

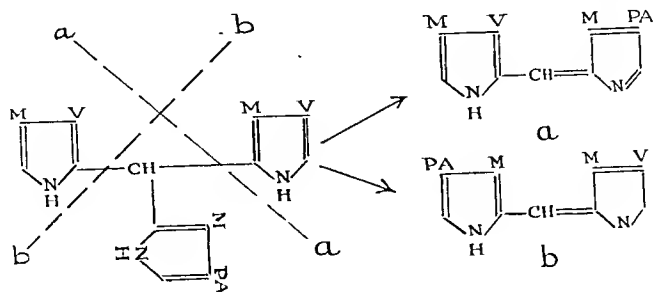


Figure VI

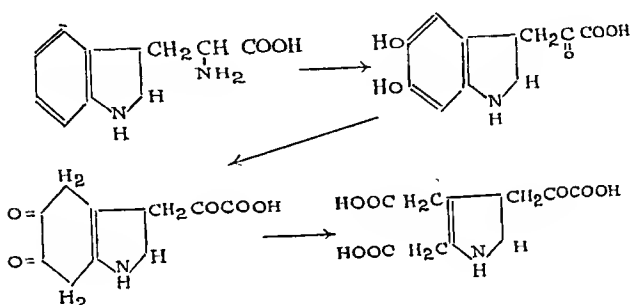


Figure VII

Finally, we may briefly consider the site of porphyrin formation. The rediscovery of cytochrome by Keilin in 1925, and the simultaneous demonstration of coproporphyrin synthesis by yeasts, reported by Fischer and his associates,³³ has been corroborated many times^{30, 35} over. It is now plain that every unicellular organism is capable of forming its own porphyrins, and there is considerable evidence that this is indeed a general as well as an ancient cellular function. This formation appears to be characteristic of growing cells, so that in an adult animal the formation of porphyrins due to the majority of cells is probably slight. In view of the great activity of the erythropoietic system in the formation of hemoglobin, it is here that the evidences for cellular porphyrinogenesis must be sought. Indeed, it is here that the most straightforward evidence is found. Repeatedly, fluorescence characteristic of the three types of porphyrins has been observed in erythroblasts, gradually fading as the addition of iron and protein dampened it.^{8, 13, 36} However, in porphyria, the frequent demonstration of zinc uroporphyrin,³¹ as well as of other porphyrins^{8, 13, 37} in the peripheral portion of liver lobules, suggests the possibility that in this condition cells other than erythropoietic may be active in porphyrinogenesis.

SUMMARY

A theory of porphyrinogenesis is proposed, based upon the postulated original formation of a 2-acetic acid, 3-propionic acid pyrrole, the products of which,

by passing through an aldehyde synthesis over a tripyrrylmethane, may form protoporphyrins 2 and 9, coproporphyrins I and III, and uroporphyrins I and III. The nature of the fundamental disturbance of metabolism in porphyria is suggested. The sources and sites of porphyrin formation are discussed.

REFERENCES

1. Günther, Hans: Die Hämatorporphyrie, *Deutsches Arch. f. klin. Med.* 105: 89, 1911.
- 2 a. Fischer, Hans: Über das Urinporphyrin, *Ztschr. f. physiol. Chem.* 95: 34, 1915.
 b. Über das Kotporphyrin, *Ibid.* 96: 148, 1915.
 c. Über das Kotporphyrin, *Ibid.* 96: 309, 1915.
 d. Die natürlichen Porphyrine und ihre Bedeutung für die Pathologie, *Wien. klin. Wchnschr.* 29: 1027, 1916.
3. Turner, W. J., and Obermayer, M. E.: Studies on Porphyria. II, *Arch. Dermat. and Syph.* 37: 549, 1938.
4. Waldenström, Jan: Studien über Porphyrie, *Acta med. Scandinav. Suppl.* 82, pp. 1-257, Stockholm, 1937.
5. Unpublished review of 230 cases by the author.
6. Turner, W. J.: Studies on Porphyria. III, *Arch. Int. Med.* 61: 762, 1938.
- 7 a. Schumm, O.: Über das "Hämatorporphyrin" aus Harn und Knochen, *Ztschr. f. physiol. Chem.* 96: 183, 1915.
 b. Über Porphyrinurie und natürliche Porphyrine, *München. med. Wchnschr.* 40: 1300, 1923.
8. Borst, M.: Untersuchungen über Porphyrie mit besonderer Berücksichtigung der congenitalen Porphyria congenita, Leipzig, 1929, S. Hirzel.
9. van der Vliet, A. J., Grotepass, W., and Revers, F. E.: Beitrag über das Porphyrin in Blut und Galle, *Klin. Wchnschr.* 11: 1534, 1932.
10. Fischer, Hans, and Orth, H.: Die Chemie des Pyrrols, Band II. Leipzig, 1937, Akadem. Verlagsgesellschaft (in particular, page 480).
11. Schreus, H. T.: Ergebnisse und Probleme der Porphyrinforschung, *Klin. Wchnschr.* 13: 121, 1934.
12. Carrié, C.: Die Porphyriae: Ihr Nachweis, ihre Physiologie und klinik, Leipzig, 1936, G. Thieme.
13. Vannotti, A.: Porphyrine und Porphyrinrankheiten, Berlin, 1937, Julius Springer.
- 14 a. Dobriner, K., Strain, W. H., and Locaho, S. A.: I. Quantitative Measurement of Coproporphyrin and Total Coproporphyrin I Excretion in Normals, *Proc. Soc. Exper. Biol. & Med.* 36: 752, 1937.
 b. Dobriner, K., and others: II. Coproporphyrin I Metabolism and Hematopoietic Activity, *Ibid.* 36: 755, 1937.
 c. Dobriner, K.: Excretion of Porphyrin by Dogs, *Ibid.* 36: 757, 1937.
 d. Dobriner, K., and Barker, W. H.: Total Coproporphyrin I Excretion in Pernicious Anemia, *Ibid.* 36: 864, 1937.
 e. Dobriner, K., and Rhoads, C. P.: The Excretion of Coproporphyrin I Following Hemorrhage in Dogs, *J. Clin. Investigation* 17: 105, 1938.
 f. Dobriner, K., Rhoads, C. P., and Hummel, L. E.: The Excretion of Porphyrin in Refractory and Aplastic Anemia, *Ibid.* 17: 125, 1938.
15. van den Bergh, A. A. Hijmans, Regniers, R., and Muller, W.: Ein Fall von kongenitaler Porphyrinurie mit Koproporphyrin im Harn und Stuhl, *Arch. f. Verdauungskr.* 42: 302, 1928.
- 16 a. Waldenström, J.: Untersuchungen über Harnfarbstoffe, hauptsächlich Porphyrine, mittels der chromatographischen Analyse, *Deutsches Arch. f. klin. Med.* 178: 38, 1935.
 b. Waldenström, J., Fink, H., and Hoerbarger, W.: Über ein neues bei der akuten Porphyrie regelmässig vorkommendes Uroporphyrin, *Ztschr. f. physiol. Chem.* 233: 1, 1935.
17. Mertens, E.: Über das Uroporphyrin bei akuter Porphyrie, *Ztschr. f. physiol. Chem.* 238: 1, 1936.
18. Fischer, H., and Libowitzky, H.: Auftreten von Uro-bzw. Koproporphyrin I bei akuter Porphyrie, *Ztschr. f. physiol. Chem.* 241: 220, 1936.
19. Dobriner, K.: Simultaneous Excretion of Coproporphyrin I and III in a Case of Chronic Porphyria, *Proc. Soc. Exper. Biol. & Med.* 35: 175, 1936.
20. Rimington, C., Roets, G. C. S., and Fourie, P. J. J.: Quantitative Studies Upon Porphyrin Excretion in Bovine Congenital Porphyrinuria (Pink Tooth). No. I. Onderstepoort J. Vet. Sci. & Animal Ind. 10: 421, 1938.
21. Unpublished studies of the author. See also Turner, W. J.: Studies on Porphyria I, *Biol. Chem.* 118: 519, 1937.
22. Mertens, E.: Über das Vorkommen von Koproporphyrin III bei chronischer kongenitaler Porphyrie, *Ztschr. f. physiol. Chem.* 258: 1, 1939.

23. Grotepass, W.: Zur Kenntnis der natürlichen Harnporphyrine, *Ztschr. f. physiol. Chem.* 253: 276, 1938.
24. Fischer, H.: Zur Frage des Dualismus des Blutfarbstoffs, *Ztschr. f. physiol. Chem.* 259: 1, 1939.
25. Rimington, C.: Some Cases of Congenita Porphyrria in Cattle, *Onderstepoort J. Vet. Sci. & Animal Ind.* 7: 567, 1936.
- 26 a. Corwin, A. H., and Andrews, J. S.: Studies in the Pyrrole Series, II. The Mechanism of the Aldehyde Synthesis of Dipyrromethenes, *J. Am. Chem. Soc.* 58: 1086, 1936. b. III. The Relation of Tripyrromethane Cleavage to Methane Synthesis, *Ibid.* 59: 1973, 1937.
27. Wrede, F., and Rothhaas, A.: Über das Prodigiosin, den roten Farbstoff des *Bacillus prodigiosus*, *Ztschr. f. physiol. Chem.* 226: 95, 1934.
28. Fischer, H., and Hofmann, H. J.: Über die Konstitution des Uroporphyrins, *Ztschr. f. physiol. Chem.* 246: 15, 1937.
29. Fischer, H., and Müller, A.: Synthese einer Tetramethyl-porphin-tetrasessigsäure. Ein Beitrag zum Konstitutionsproblem des Uroporphyrins, *Ztschr. f. physiol. Chem.* 246: 31, 1937.
30. Coulter, C. B., and Stone, F. M.: The Identification of the Porphyrin Compound Found in Cultures of *C. diphtheriae* and *Mycobacteriae*, *Proc. Soc. Exper. Biol. & Med.* 38: 423, 1938.
31. Unpublished studies of the author on autopsy material from latent porphyria and personal communication from Dr. C. J. Watson.
32. Thomas, J.: Contribution à l'étude des porphyrines en biologie et en pathologie. I. Genèse du noyau tétrapyrrolique, *Bull. Soc. Chim. Biol.* 20: 471, 1938.
- 33 a. Fischer, H., and Hilger, J.: Zur Kenntnis der natürlichen Porphyrine. X. Blutfarbstoff in der Hefe, Nachweis von Porphyrin in Pflanzen, *Ztschr. f. physiol. Chem.* 138: 288, 1924.
b. Fischer, H., and Fink, H.: Über Koproporphyrinsynthese durch Hefe und ihre Beeinflussung, *Ibid.* 150: 243, 1925.
34. Keilin, D.: On Cytochrome, a Respiratory Pigment, Common to Animals, Yeast, and Higher Plants, *Proc. Roy. Soc., London*, s. B. 98: 312, 1925.
35. Jakob, A.: Über den Abbau von Blutfarbstoff zu Porphyrinen durch Reinkulturen von Bakterien und über eine neue biologische Synthese von Koproporphyrin III, *Klin. Wchnschr.* 18: 1024, 1939.
36. Emminger, E., and Battistini, G.: Fluoreszenzmikroskopische Untersuchungen an bleivergifteten Kaninchen mit und ohne Calciumzufuhr, *Virchows Arch. f. path. Anat.* 290: 492, 1933.
37. Emminger, E.: Fluoreszenzmikroskopische Untersuchungen bei einem Fall von Schlafmittelvergiftung, *Klin. Wchnschr.* 12: 1840, 1933.

STUDIES ON THE INTRAVENOUS INJECTION OF COLLOIDS*

II. EFFECTS OF GUM ACACIA ON CERTAIN FUNCTIONS OF THE LIVER WITH A NOTE ON ITS EFFECTS ON THE PRODUCTION OF IMMUNE BODIES

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ANDERSCH and Gibson¹ in 1934 demonstrated in a series of experimental observations that a large part of the gum acacia injected intravenously in rabbits and in dogs was not excreted, as had been generally assumed, but was deposited in the hepatic cells. An average of 30 and 33 per

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cent, respectively, was found in the livers of two dogs studied, and an average of 50 per cent in the livers of rabbits. Histologic examination of the liver tissue, and marked changes in the composition of the bile, especially of the bile pigment excreted by the dogs under observation, led these authors to conclude that intravenous injections of gum acacia were likely to cause marked liver damage. In the same contribution they reported a fatal clinical case of nephrosis in which this form of therapy had been employed and in which 43 per cent of the injected acacia was found in the liver. The liver cells presented the same characteristic vacuolated appearance noted in the liver cells of the experimental animals.

In the following year Dick, Warweg, and Andersch² extended these clinical and experimental observations and concluded that the intravenous use of gum acacia in patients with nephrosis was likely to be attended with harmful effects.

In 1937 Studdiford¹ called attention to the extreme danger of employing intravenous injections of gum acacia in patients with already damaged livers, such as are likely to be found in the toxemias of pregnancy and similar disorders. This form of therapy, he pointed out, might interfere with the gaseous exchanges of the red blood cells, and if carried to a marked degree, might produce severe or fatal anoxemia, especially if faulty solutions were employed. He also produced evidence to show that such solutions might cause "conglutination of the red cells, with resultant capillary blockage followed by edema and hemorrhage."

The purpose of the present contribution is twofold: to study liver function by various tests after the experimental intravenous injection of acacia, and to observe the possible effects of this method of treatment on the production of immune bodies. The general procedures employed in the handling of the experimental animals and the injection of the gum acacia, together with the methods of analysis used for acacia, are set forth in detail by Andersch and Gibson.¹

STUDIES IN LIVER FUNCTION

Glucose and Galactose Tolerance Tests.—Glucose and galactose tolerance tests were performed on normal dogs after they had fasted eighteen hours or more. Blood for a control sugar determination⁴ was taken from the marginal vein of the ear, and other samples were taken every half hour for a two-hour period following the administration by stomach tube of 1.5 Gm. of sugar per kilogram of body weight (Table I). The blood galactose,⁵ as well as the total sugar, was determined subsequently in two more dogs in the course of the galactose tolerance tests. All the dogs were then given from three to seven intravenous injections of gum acacia (totaling 4.4 to 10.7 Gm. per kilogram of body weight) over periods varying from three days to four weeks. Several days after the last acacia injection the glucose and galactose tolerance tests were repeated.

The control glucose curves obtained in this study are similar to those obtained by Bodansky⁶ after the oral administration of sugar, and by Soskin, Allweiss, and Cohn⁷ after its intravenous administration. The curves ob-

tained after the injections of acacia apparently correspond with the glucose tolerance curves obtained by Soskin and Mirsky⁸ in severe liver intoxications.

The galactose blood clearance controls agree with the curves obtained on normal dogs by Bodansky.⁶ A marked diminution in galactose tolerance was observed after the injection of acacia.

TABLE I

EFFECT OF INTRAVENOUS INJECTIONS OF ACACIA UPON TOLERANCE OF DOGS
TO GLUCOSE AND GALACTOSE

DOG NO.	ACACIA TOTAL GM./KG.	GLUCOSE TOLERANCE BLOOD SUGAR AT HALF HOURS MG. PER CENT OF GLUCOSE							GALACTOSE TOLERANCE BLOOD SUGAR AT HALF HOURS MG. PER CENT AS GLUCOSE							
		0.0	0.5	1.0	1.5	2.0	2.5	3.0	0.0	0.5	1.0	1.5	2.0	2.5	3.0	
1	0	100	147	107		81			92	157	145	131	129			
	4.4	120	157	157	140	98			98	200	236	212	162			
2	0	81	118	107	82	79			81	162	165	192	173			
	7.5	69	165	179	138	79			90	131	189	231	175			
4	0	50	150	122	80	78			87	135	122	96	85			
	9.0	80	170	147	90	95			90	142	186	157	102			
5	0	92	179	173	90	74			120	167	212	150	142			
	10.7	96	217	212	165	96			102	192	226	212	167			
6	0								79	167	186	159	129			
	8.4								86	186	226	182	138			
10		GALACTOSE TOLERANCE BLOOD SUGAR AT HALF HOURS MG. PER CENT OF GALACTOSE														
	0		80*	112	100	68			80	143	170	156	139			
	8.5		59	73	97	81	45		90	142	155	172	138	125		
11	0		71*	91	122	109	31		91	154	167	200	183	107		
	6.8		101	161	164	151	66	35	85	160	221	225	223	133	113	

*The total sugar values obtained in these tests are reported on the same line on the right side of the table.

Tetraiodophenolphthalein and Bilirubin Tests.—Dogs were tested by a modification of the sodium tetraiodophenolphthalein liver function procedure of Graham.⁹ Ninety seconds after the injection into the jugular vein of 25 mg. per kilogram body weight of tetraiodophenolphthalein (isoiodeikon), a sample of blood was withdrawn from the vein on the opposite side and oxalated. Additional samples of blood were obtained fifteen, thirty, and sixty minutes later. One cubic centimeter of plasma from each specimen was made alkaline with 2 drops of 10 per cent sodium hydroxide, 10 c.c. of ethyl alcohol were added, the contents of each tube were mixed, placed in the ice-box overnight, and centrifuged in the morning. The test of the first blood sample was used as a standard for the colorimetric determination of the dye in the subsequent specimens.

Observations on four dogs revealed no significant changes in the ability of the liver to excrete the dye before and after injections of gum acacia; in two other dogs there was apparently a slight reduction.

For the bilirubin test the method suggested by Soffer¹⁰ was employed. One milligram of bilirubin per kilogram of body weight was given intravenously to two normal dogs and to two dogs which had had intravenous

injections of acacia (Table II). Samples of blood were withdrawn before injection of the pigment, and again five minutes and four hours afterward. The plasma bilirubin in the specimens was determined quantitatively by the Gibson and Goodrich¹¹ modification of the van den Bergh procedure. The Soffer method, which was suggested for clinical use, gave a rather low degree of bilirubinemia. We therefore repeated the test, using the procedure suggested by Dragstedt and Mills.¹² Ten milligrams of bilirubin per kilogram of body weight were injected intravenously, and blood samples were withdrawn before the injection, and five minutes, thirty minutes, one hour, and two hours afterward. In none of the four dogs used was there any alteration in the rate of removal of the injected bilirubin.

TABLE II

BILIRUBIN LIVER FUNCTION TEST ON NOEMAL DOGS AND DOGS AFTER ACACIA INJECTIONS

DOG NO.	WEIGHT (KG.)	ACACIA INJECTED (GM.)	BILIRUBIN INJECTED (MG.)	VAN DEN BERGH—MG. BILIRUBIN						
				TIME IN MINUTES						
				0	2.5	5	30	60	120	240
3	9.5	0	10.0	0.2		1.2				0.4
5	8.7	96.6	8.7	0.6	1.9	1.3				0.7
6	8.0	67.5	8.0	0.5		0.7				0.0
7	9.2	0	9.2	0.3	1.9	2.0				0.6
5	8.7	96.6	87.0	0.2		15.3	5.0	2.9	2.1	
7	9.2	0	92.0	0.3		15.0	5.3	2.8	2.5	
8	5.5	0	55.0	0.2		7.0	3.7	1.4	1.0	
8	5.5	41.4	55.0	0.2		4.0	1.8	1.0	0.9	
9	5.0	0	50.0	0.3		6.5	3.9	1.4	0.9	
9	5.0	45.0	50.0	0.4		6.8	2.7	0.9	0.9	

TABLE III

EFFECT OF INTRAVENOUS ACACIA INJECTIONS ON PLASMA PROTEIN LEVEL

DOG NO.	WEIGHT (KG.)	PLASMA PROTEIN IN GRAMS PER CENT								REMARKS
		ALBUMIN		GLOBULIN		FIBRINOGEN		TOTAL PROTEIN		
		BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER	
2	9.7	2.94	2.54	1.66	1.86	0.38	0.14	4.98	4.54	82.5 Gm. acacia blood drawn 4 days after last acacia injection
4	7.5	2.52	2.11	2.74	2.58	0.41	0.24	5.67	4.93	67.5 Gm. acacia blood drawn 3 days after last acacia injection
5	8.7	2.44	1.72	1.96	1.10	0.32	0.04	4.70	2.86	96.6 Gm. acacia blood drawn 4 days after last acacia injection
6	8.0	2.74*	2.20	2.03*	1.80	0.37*	0.21	5.14*	4.20	67.5 Gm. acacia blood drawn 5 days after last acacia injection

*This sample of blood was drawn a few minutes after the first acacia injection.

Plasma Protein Determinations.—Plasma protein determinations were made by the colorimetric method of Andersch and Gibson,¹³ modified by washing the fibrinogen precipitate and substituting the tyrosine factors

tained after the injections of acacia apparently correspond with the glucose tolerance curves obtained by Soskin and Mirsky⁸ in severe liver intoxications.

The galactose blood clearance controls agree with the curves obtained on normal dogs by Bodansky.⁶ A marked diminution in galactose tolerance was observed after the injection of acacia.

TABLE I

EFFECT OF INTRAVENOUS INJECTIONS OF ACACIA UPON TOLERANCE OF DOGS TO GLUCOSE AND GALACTOSE

DOG NO.	ACACIA TOTAL GM./KG.	GLUCOSE TOLERANCE BLOOD SUGAR AT HALF HOURS MG. PER CENT OF GLUCOSE							GALACTOSE TOLERANCE BLOOD SUGAR AT HALF HOURS MG. PER CENT AS GLUCOSE							
		0.0	0.5	1.0	1.5	2.0	2.5	3.0	0.0	0.5	1.0	1.5	2.0	2.5	3.0	
1	0	100	147	107		81			92	157	145	131	129			
	4.4	120	157	157	140	98			98	200	236	212	162			
2	0	81	118	107	82	79			81	162	165	192	173			
	7.5	69	165	179	138	79			90	131	189	231	175			
4	0	50	150	122	80	78			87	135	122	96	85			
	9.0	80	170	147	90	95			90	142	186	157	102			
5	0	92	179	173	90	74			120	167	212	150	142			
	10.7	96	217	212	165	96			102	192	226	212	167			
6	0								79	167	186	159	129			
	8.4								86	186	226	182	138			
GALACTOSE TOLERANCE BLOOD SUGAR AT HALF HOURS MG. PER CENT OF GALACTOSE																
10	0		80*	112	100	68			80	143	170	156	139			
	8.5		59	73	97	81	45		90	142	155	172	138	125		
11	0		71*	91	122	109	31		91	154	167	200	183	107		
	6.8		101	161	164	151	66	35	85	160	221	225	223	133	113	

*The total sugar values obtained in these tests are reported on the same line on the right side of the table.

Tetraiodophenolphthalein and Bilirubin Tests.—Dogs were tested by a modification of the sodium tetraiodophenolphthalein liver function procedure of Graham.⁹ Ninety seconds after the injection into the jugular vein of 25 mg. per kilogram body weight of tetraiodophenolphthalein (isoiodeikon), a sample of blood was withdrawn from the vein on the opposite side and examined. Additional samples of blood were obtained fifteen, thirty, and sixty minutes later. One cubic centimeter of plasma from each specimen was made alkaline with 2 drops of 10 per cent sodium hydroxide, 10 c.c. of ethyl alcohol were added, the contents of each tube were mixed, placed in the ice-box overnight, and centrifuged in the morning. The test of the first blood sample was used as a standard for the colorimetric determination of the dye in the subsequent specimens.

Observations on four dogs revealed no significant changes in the ability of the liver to excrete the dye before and after injections of gum acacia; in two other dogs there was apparently a slight reduction.

For the bilirubin test the method suggested by Soffer¹⁰ was employed. One milligram of bilirubin per kilogram of body weight was given intravenously to two normal dogs and to two dogs which had had intravenous

almost specifically utilized by the liver. The galactose curves are more decisive, and the diminution of galactose tolerance noted after the injection of acacia undoubtedly indicates liver damage. The impairment of glycogenic function after acacia injection is also borne out by post-mortem observations on liver glycogen made on three dogs. The livers contained respectively 6.6, 7.7, and 6.9 per cent of glycogen, an average of 7.1 per cent against the normal value of 15 per cent to be expected in dogs fed, as these were, a high carbohydrate diet. Bollman and Mann¹⁸ have observed that dogs with 80 per cent of the liver removed are still able to take care normally of glucose and galactose.

The figures which Soffer¹⁰ gives in an extensive review (including his own work) show that an abnormal retention of bilirubin is more closely correlated with the degree of liver damage than are the bromsulphalein or the levulose tolerance tests of liver function. We found no significant alteration in the rate of removal of injected bilirubin before and after the injection of gum acacia, and only a slight variation in two dogs in the excretion of tetraiodophenolphthalein. It seems logical to conclude, therefore, that this special function of the liver is not affected by the injection of acacia.

The experimental studies of Whipple and his co-workers¹⁹⁻²¹ point to the liver as the source of serum albumin and globulin. A number of studies by others²²⁻²⁶ are concerned with the effect of severe liver disease on the plasma proteins, particularly papers by Snell²⁵ and by Myers and Keefer.²⁶ These bear out Snell's conclusion that in advanced chronic hepatic lesions there is "a moderate reduction in the total serum proteins, the diminution occurring chiefly in the albumin fraction." Dick, Warweg, and Andersch² have shown that repeated injections of acacia in children with nephrosis resulted in a reduction of blood serum protein, chiefly albumin. They also found that daily injections of acacia into dogs caused a sharp drop in the serum albumin and a less marked fall in the globulin, both values returning to normal when the injections were discontinued. Our own observations are in accord with these results, as are the more recent studies of Heckel and his associates.²⁷

Foster and Whipple²⁸ have shown that fibrinogen is formed exclusively by the liver. Low values may be found in hepatic disease, though the picture may be complicated by the stimulating effects of infection on fibrinogen production. The effect on fibrinogen levels of various clinical and experimental conditions involving the liver has been reviewed by Starlinger and Winands²⁹ and by Ham and Curtis.³⁰ It may be said that complete extirpation of the liver in dogs³¹ and in rabbits³² results in a rapid fall in plasma fibrinogen, in some experiments amounting to 50 per cent in twenty-four hours; removal of as much as 70 per cent of hepatic tissue, on the other hand, has no effect on the fibrinogen level.

Foster and Whipple,³³ who found lowered blood fibrin values after the injection of acacia, ascribed the reduction to dilution of the blood. The possible influence of gum-saline solutions on blood coagulation is mentioned by Takata³⁴ and others. Dieckmann,³⁵ who tried gum acacia injections in the treatment of eclampsia, specifically warns that this form of therapy may cause a pronounced drop in blood fibrin. The decrease in hemoglobin, the increased bleeding time, and the low fibrinogen values noted in our experimental animals after the injection

TABLE V
PRODUCTION OF IMMUNE BODIES IN NORMAL DOGS AND IN DOGS WHICH HAD HAD ACACIA INJECTIONS

DOG NO.	WEIGHT (KG.)	GRAMS OF ACACIA PER KG.	TITER OF AGGLUTININ TO VIBRIO CHOLERA		TITER OF PRECIPITIN TO VIBRIO CHOLERA		TITER OF AGGLUTININ TO ERYTHROCYTES		TITER OF PRECIPITIN TO HEMOLYZED ERYTHROCYTES		TITER OF HEMOLYSIS	
			BEFORE IMMUNIZATION	AFTER IMMUNIZATION	BEFORE IMMUNIZATION	AFTER IMMUNIZATION	BEFORE IMMUNIZATION	AFTER IMMUNIZATION	BEFORE IMMUNIZATION	AFTER IMMUNIZATION	BEFORE IMMUNIZATION	AFTER IMMUNIZATION
2	9.7	7.3	0	$\frac{1}{2,560}$	0	$\frac{1}{12,800}$	0	$\frac{1}{640}$	0	$\frac{1}{204,800}$	$\frac{1}{4}$	$\frac{1}{50}$
3	9.5	0	0	$\frac{1}{5,120}$	0	$\frac{1}{12,800}$	0	$\frac{1}{12,800}$	0	$\frac{1}{204,800}$	$\frac{1}{4}$	$\frac{1}{200}$
5*	8.7	10.7	0	$\frac{1}{320}$	0	$\frac{1}{200}$	0	$\frac{1}{2,560}$	$\frac{1}{200}$	$\frac{1}{1,600}$	0	$\frac{1}{40}$
6*	8.0	8.4	0	$\frac{1}{320}$	0	0	0	$\frac{1}{2,560}$	0	$\frac{1}{1,600}$	$\frac{1}{10}$	$\frac{1}{40}$
7*	9.2	0	0	$\frac{1}{1,280}$	0	0	0	$\frac{1}{2,560}$	0	$\frac{1}{3,200}$	$\frac{1}{10}$	$\frac{1}{40}$

*The titration of antibodies "before immunization" with Dogs 5, 6, and 7 was done on blood drawn three days after the first injection of antigen. Dogs 3 and 7 are normal animals.

tion of gum acacia are undoubtedly significant and would seem to indicate a rather severe degree of liver damage. Additional confirmation is found in the death of one dog from a postoperative hemorrhage after several injections of gum acacia.

EFFECT OF COLLOIDS ON THE PRODUCTION OF IMMUNE BODIES

The injection of various colloids has been shown to result in a marked reduction in the ability of the animal to produce immune bodies.³⁶ These studies have led to an association of the reticulo-endothelial system with antibody production. However, the hepatic cells of the liver have never been definitely excluded from a role in antibody formation. The plasma proteins which carry the antibodies of the blood have their origin in the liver, and the ability of the organism to produce these proteins is impaired by the acacia which is deposited in the hepatic cells. Acacia itself is at most only a very mild antigen.^{37, 38}

In order to determine the effect of acacia injections upon the ability of the dog to produce immune bodies, five animals, of approximately equal weight, were used. Three of them (Nos. 2, 5, and 6) had had intravenous gum injections and had been tested by various liver function tests (Tables I, III, and IV). The other two (Nos. 3 and 7) were used as controls.

Two antigens were used. The first was a 5 per cent suspension of pooled washed human erythrocytes. The second was a vaccine of *Vibrio cholera* made from whole and ground organisms which had been killed by phenyl mercuric nitrate, 0.12 per cent; the cholera was strain No. 23 of the American Type Culture Collection. Five cubic centimeters of the suspension of human red blood cells were injected intravenously every three days until five injections had been given; 0.1 c.c. of the cholera vaccine was injected intravenously with the erythrocyte suspension for three days, and 0.2 c.c. for the last two days. Eight days after the final injection of the antigens the animals were bled and the titer of agglutinins, precipitins, and bacteriolysins or hemolysins was determined. Samples of blood drawn before immunization were also checked for the presence of antibodies.

The presence of cytotoxins for *Vibrio cholera* could not be demonstrated in either the normal or the immune sera. Hemolysin titrations were not very satisfactory, and the titers produced were low. Differences between the titers of antibody produced by normal dogs and those receiving acacia were not significant, and the data all indicate that gum acacia has no effect upon the production of immune bodies (Table V). This lack of effect is in marked contrast to the reduction of antibody formation likely to result when colloids which definitely block the reticulo-endothelial system are injected.

SUMMARY AND CONCLUSIONS

1. Repeated intravenous injections of gum acacia solution in dogs resulted in evident damage for carbohydrate and serum protein metabolism functions of the liver, as evidenced by changes in the glucose and galactose blood sugar tolerance curves and determination of the plasma proteins.

2. The blood clearance of injected tetraiodophenolphthalein and bilirubin was not significantly affected.

BLOOD SERUM MAGNESIUM IN BRONCHIAL ASTHMA AND ITS TREATMENT BY THE ADMINISTRATION OF MAGNESIUM SULFATE*

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A NUMBER of investigators have recently shown that magnesium salts will protect animals against anaphylactic shock. Delbet and Palios¹ have found that a sensitized guinea pig may be protected against the provocative injection of the specific antigen by parenteral injections of magnesium salts. Lumière and Monchal² have also found that sensitized rabbits may be protected by one or more daily injections of 0.1 Gm. per kg. of magnesium thiosulfate. They observed that such injections of the thiosulfate produced a 50 to 100 per cent increase in blood cholesterol and concluded that the antishock action of this salt was due entirely to the cholesterolemia it produced. I³ have observed, however, that if the serum magnesium of sensitized guinea pigs is increased to about 8 mg. per 100 c.c. immediately before the provocative injection of the antigen is made, anaphylactic shock may be prevented. This would seem to indicate that hypermagnesemia rather than a cholesterolemia is the protective agent. If hypermagnesemia will prevent anaphylactic shock, we may hypothesize that hypomagnesemia may make one more sensitive to, or even incite an anaphylactic reaction.

The similarity between the bronchial spasms in anaphylactic shock and bronchial asthma is now generally conceded. The possibility that a hypomagnesemia may be an etiologic factor in bronchial asthma naturally suggested itself. The findings of Rosella and Plá⁴ that intravenous injection of magnesium sulfate relieved a case of asthma which was unaffected by the common remedial agents, adds an additional premise to this theory. ✓

The present investigation was undertaken to determine, if possible, if patients suffering from bronchial asthma have a concomitant hypomagnesemia.

METHOD

Sixty-six asthmatic patients were chosen at random as admitted to the Allergy Clinic and the Medical Wards of the Jefferson College Hospital.†

About 10 c.c. of blood were collected from each patient from the median basilic vein. Serum magnesium determinations were made by the Titan yellow method described elsewhere.⁵ In the hospitalized patients a series of determi-

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nations were made at daily intervals. The serum magnesium values of 40 normal medical students were previously ascertained. As the ingestion of food has very little influence on the concentration of serum magnesium, no effort was made to obtain blood at any specified period in relation to meals. All determinations were made in duplicate.

RESULTS

Normal Persons.—Forty determinations were made, using blood obtained from students in the medical school. The values obtained varied between 1.74 and 3.10 mg. per 100 c.c. serum, with a mean value of 2.33 mg. These figures are in accordance with those found by Beecher,⁶ Bomskov,⁷ Waeker and Fahrig,⁸ Walker and Walker,⁹ and Hirschfelder and myself.¹⁰

Serum magnesium values below 1.7 mg. per 100 c.c. serum are, therefore, considered as being definitely below normal. Values above 3.1 mg are considered as being high.

Asthmatic Subjects.—The serum magnesium of the 66 individuals suffering from bronchial asthma varied between 1.1 and 3.15 mg. per 100 c.c., with a mean value of 2.13 mg. Of the entire group of 66 individuals 16 (24.2 per cent) had a low serum magnesium, varying from 1.1 to 1.7 mg. per 100 c.c., with a mean value of 1.52 mg. Twenty-six of the patients examined were suffering an acute attack of asthma at the time the blood samples were taken. In these the serum magnesium values varied between 1.1 and 3.1 mg per 100 c.c., with a mean value of 1.82. The remaining 40 individuals were free of asthma at the time the blood was analyzed. In these the mean magnesium value was 2.32 mg. per 100 c.c. serum (see Table I).

Upon further analysis of the data, one finds that of the 26 patients having acute asthmatic spasms, 13 (50 per cent) had a concomitant hypomagnesemia. In this latter group the serum magnesium varied between 1.10 and 1.70 mg., with a mean of 1.50 mg. per 100 c.c. The serum magnesium of the 13 remaining cases was entirely within normal range.

The Effect of Injections of Magnesium Sulfate.—Two patients suffering from severe asthma were treated with intravenous and intramuscular injections of magnesium sulfate. The results obtained were essentially the same as those reported by Rosella and Plá.⁴

Two white male patients, aged 39 and 56 years, respectively, hospitalized at the Jefferson Medical College Hospital, were suffering with acute bronchial asthma. Both patients had a low serum magnesium, averaging 1.53 and 1.60 mg. per 100 c.c., respectively, over a four-day period. Epinephrine administration was required every four to six hours, but on several occasions this drug was ineffective. This form of treatment was discontinued, and at the height of the asthmatic paroxysms 20 c.c. of 10 per cent magnesium sulfate were injected slowly intravenously and 4 c.c. of the 50 per cent solution were given deep intramuscularly. Both patients were relieved immediately and remained free of asthma for eighteen and twenty-eight hours, respectively. Following the reappearance of the bronchial spasms, epinephrine therapy was again instituted, requiring as before, an injection every four to six hours.

DISCUSSION

The majority of patients examined did not have any attacks of asthma for a period of from one day to more than three months previous to the time the blood was examined. It is to be expected, therefore, that normal values should be found in these cases. It seems significant, however, that one-half of the cases examined during acute attacks of asthma should be found to have a definite hypomagnesemia. It appears even more significant that of the 16 individuals having a low serum magnesium, 13 should be suffering from an acute attack of asthma. The degree of hypomagnesemia, however, was not in proportion to the severity of the attacks. Patients S. R. and C. P. with relatively mild asthma were found to have magnesium values of 1.20 and 1.35 mg. per 100 c.c. serum, respectively. Patients E. S. and J. Q., on the other hand, suffered severe and persistent attacks, although the magnesium content of their blood was 1.53 and 1.60, respectively. Three individuals with a hypomagnesemia did not have any attacks for 2, 6, and 21 days, respectively.

That 50 per cent of the patients having acute attacks of asthma should have a low serum magnesium indicates that a hypomagnesemia and bronchial asthma may have some relationship. It appears quite plausible, at least in some cases, that a deficiency of this ion may upset the neuromuscular mechanism to such an extent as to make certain individuals more susceptible to bronchial spasms. It has been conclusively shown by Kruse and his collaborators^{11, 12} and others¹³⁻¹⁶ that such an ionic deficiency manifests itself chiefly by muscular spasms and convulsions. We must remain cognizant, however, of the possibility that the hypomagnesemia may be the result of the bronchial asthma.

Of equal importance is the fact that 50 per cent of the patients examined during attacks of asthma had normal serum magnesium. This means that a hypomagnesemia is by no means an essential etiologic factor. At present we may conclude that a disturbance in magnesium metabolism may be a contributing factor in a certain percentage of patients suffering from bronchial asthma. Further investigations in this direction should prove most interesting.

SUMMARY

1. The serum magnesium of 40 normal medical students varied between 1.74 and 3.10 mg. per 100 c.c., with a mean value of 2.33 mg.
2. The blood sera of 66 patients suffering with bronchial asthma were analyzed for their magnesium content with an analysis of the results.
3. The treatment of two patients with parenteral injections of magnesium sulfate is reported.

REFERENCES

1. Delbet, P., and Palios: Halogen Salts of Magnesium as Antianaphylactics, An Experimental Study, *Bull. Acad. de méd.*, Paris 117: 96, 1937.
2. Lumière, A., and Monchal, S.: Action of Intravenous Injections of Magnesium Thiosulfate on Cholesterol Content of Blood Studied in Relation to Its Use in Protection Against Anaphylactic Shock, *Compt. rend. Soc. de biol.* 124: 178, 1937.
3. Haury, V. G.: Unpublished observations.
4. Rosella, J. C., and Plá, J. C.: Magnesium Sulfate in Crisis of Asthma, *Prensa méd. argent.* 23: 1677, 1936.

5. Haury, V. G.: Modification of the Titan Yellow Method for the Determination of Small Amounts of Magnesium in Biological Fluids, *J. LAB. & CLIN. MED.* 23: 1679, 1938.
6. Becher, E.: Der Magnesium—Gehalt des Blutes unter normalen und pathologischen Verhältnissen, insbesondere bei Nierenkrankheiten, *Klin. Wehnschr.* 11: 202, 1932.
7. Bomskov, C.: Determination of Magnesium in Blood With 8-hydroxyquinoline, *J. Biol. Chem.* 99: 17, 1932.
8. Wacker, L., and Fahrig, C.: Über die mineralischen und lipoiden Bestandteile des Blutserums bei der essentiellen Hypertension im Vergleich zu den physiologischen Verhältnissen, *Klin. Wehnschr.* 11: 762, 1932.
9. Walker, B. S., and Walker, E. W.: Normal Magnesium Metabolism and Its Significant Disturbances, *J. LAB. & CLIN. MED.* 21: 713, 1936.
10. Hirschfelder, A. D., and Haury, V. G.: Variations in Magnesium and Potassium Associated With Essential Epilepsy, *Arch. Neurol. & Psychiat.* 40: 66, 1938.
11. Kruse, H. D., Orent, E. E., and McCollum, E. V.: Studies on Magnesium Deficiency in Animals, I. Symptomatology Resulting From Magnesium Deprivation, *J. Biol. Chem.* 96: 519, 1932.
12. Orent, E. R., Kruse, H. D., and McCollum, E. V.: Studies on Magnesium Deficiency in Animals, II. Species Variation in Symptomatology of Magnesium Deprivation, *Am. J. Physiol.* 101: 454, 1932.
13. Metzger, H. J.: A Case of Tetany With Hypomagnesemia in a Dairy Cow, *Cornell Veterinarian* 26: 353, 1936.
14. Hopkirk, C. S. M.: Magnesium in "Grass Staggers," New Zealand Department of Agriculture, Annual Report, 1934-35.
15. Cunningham, I. J.: The Distribution of Magnesium in the Animal Organism and the Effect of Dietary Magnesium, *New Zealand J. Sc. Tech.* 18: 419, 1936.
16. Seekles, L., and Sjöellema, B.: Etiology of "Grass" Tetany, *Arch. Tierheilk.* 68: 356, 1935.

SODIUM AND POTASSIUM STUDIES IN PERSONS WITH AND WITHOUT ARTERIAL HYPERTENSION*

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THE relation of sodium and potassium to arterial blood pressure has not been studied extensively. It is, of course, well known that in adrenal cortical insufficiency (Addison's disease) hypotension and a low serum sodium and a high serum potassium occur; and conversely, in the hyperadrenal-cortical state the opposite is observed, namely, hypertension and a high serum sodium and low serum potassium.^{1, 2} There is, however, no evidence to show that the altered sodium and potassium levels are directly related to the altered state of the blood pressure. Furthermore, McCance³ has pointed out that the findings of a low serum sodium associated with a high blood pressure are quite common in interstitial nephritis.

The following study has been carried out to determine, if possible, whether the subject with arterial hypertensive disease might reveal some evidence of a disturbance in sodium and potassium metabolism when these two elements were drastically restricted in the diet.

A group of five male subjects was placed on a diet low in sodium and potassium content. Three of the subjects studied (J. L. B., aged 70 years; J. F. W., aged 58 years; and W. L. S., aged 28 years) had had definite elevation of systolic and diastolic blood pressures of from one to twelve years' duration, with

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or without evidence of renal disease but without evidence of abnormal retention of fluid. None of the subjects gave evidence of nitrogen retention in the blood.

The two persons in the control group (W. Y. B., aged 21 years and J. A. N., aged 24 years) had normal blood pressure readings and no evidence of fluid retention. W. Y. B. had an anxiety neurosis, and had had before the study was undertaken a systolic blood pressure reading as high as 150. His diastolic blood pressure was at all times within normal limits. Immediately before and during the study his systolic blood pressure was within normal limits.

All patients were confined to the hospital for observation for periods of from two to fifteen days before the study was begun. During the study their physical activities were restricted to such exercise as walking about the ward. At no time was abnormal sweating present. The environmental temperature was fairly constant, maintained at approximately 22° C. During a preliminary period of twenty-four hours, the fluid intake, urinary output, blood pressure, and body weight were determined. At the end of this period venous blood was obtained after the patient had been fasting for fourteen hours, for determination of the serum sodium, potassium, and protein; the plasma nonprotein nitrogen; and the blood sugar, chlorides, and carbon dioxide (see Table II and Chart 1).

The subjects were then given a special diet, which had been prepared under the supervision of Miss A. Huesman, of the Dietetic Department of the University of California Hospital.

SPECIAL MIXTURE*

Daily portion:

125 Gm. of dry uncooked white rice

125 Gm. of washed cottage cheese

10 Gm. of pure glucose

Method of preparation:

Wash rice thoroughly; drain. Mix rice with 1½ quarts of distilled water. Place in heavy kettle over direct heat. Boil for twenty minutes; drain in sieve. Mix rice that has been drained with 1 quart of distilled water and boil for ten minutes. Drain. Repeat last process and drain.

Purée commercially prepared cottage cheese through very fine strainer. Place in cheesecloth bag. Wash in 2 quarts tap water, kneading thoroughly; drain. Repeat process eight times. Repeat ninth time using distilled water. Drain overnight. Weigh 125 Gm. of washed cottage cheese.

Mix together cooked rice, washed cottage cheese, and glucose that has been dissolved in ½ cup distilled water. To this mixture add distilled water until the total weight of mixture equals 810 Gm.

Food value:

Protein 25 Gm.

Fat 2 Gm.

Carbohydrate 113 Gm.

Total number of calories 610

A sample of each batch of food so prepared was analyzed for the sodium and potassium content (see Chart 1).

The fluid intake, exclusive of the diet described above, was limited to 1,000 c.c. of distilled water in twenty-four hours. It was found that this amount of

*A sufficient amount of food for each subject was made at one time for the duration of the test.

fluid satisfied the thirst requirement while taking the diet. Occasionally a patient would take even less than the amount permitted (see Table I).

All the determinations of sodium and potassium were made, using the method described by Butler and Tuthill⁴ for the sodium, and that described by Shohl and Bennett⁵ for the potassium with the modifications suggested by Strauss.⁶ The urine sodium determinations were made as described by Butler and Tuthill⁴ without modifications in technique. The other specimens for sodium and potassium determinations were first dried on a hot plate and then ashed in a muffle furnace at 600° C. for approximately eight hours. In collecting all the specimens, care was exerted to maintain a uniform procedure. No evidence of hemolysis was observed in any serum specimens.

TABLE I

EFFECT OF LOW SODIUM AND POTASSIUM INTAKE ON BLOOD PRESSURE, BODY WEIGHT, FLUID BALANCE, AND URINE

		NORMAL FASTING	24 HOURS LOW Na AND K REGIME FASTING	48 HOURS LOW Na AND K REGIME FASTING	72 HOURS LOW Na AND K REGIME FASTING	96 HOURS LOW Na AND K REGIME + 6 GM. KCl FASTING	120 HOURS LOW Na AND K REGIME + 6 GM. KCl FASTING
Blood pressure in mm. Hg	J. L. B.	200/95	180/80	180/80	180/85		
	W. Y. B.	140/80	120/75	130/70	125/70		
	J. F. W.	225/135	215/125	200/120	195/120	190/115	190/115
	W. L. S.*	125/95	145/95	135/90	145/100	120/85	125/90
	J. A. N.	130/75	125/70	120/70	130/70	120/65	125/70
Urine output c.c.	J. L. B.	1,355/1,000	501/400	369/400	264/700		
	W. Y. B.	1,848/2,150	1,368/1,000	720/850	1,297/1,000		
Fluid intake c.c. (Exclusive of diet)	J. F. W.	1,800/1,490	1,475/1,000	1,447/1,000	1,055/900	587/650	710/700
	W. L. S.	860/1,100	1,165/900	1,205/800	850/800	950/1,000	1,267/1,000
	J. A. N.	4,845/5,550	1,840/1,000	545/1,000	795/1,000	530/1,000	680/1,000
Body weight in kg.	J. L. B.	67	67	66.8	66.2		
	W. Y. B.	66.1	64.7	64.1	63.1		
	J. F. W.	58.4	58.2	57.4	56.4	56.4	56
	W. L. S.	60.2	59.4	59	58.4	57.4	56.8
	J. A. N.	69	67	66.3	66	65.3	65.3
Urine pH	J. L. B.	-/1.014	-/1.029	-/1.028	-/1.022		
Urine specific gravity	W. Y. B.	-/1.016	-/1.014	5.5/1.020	5.5/1.013		
	J. F. W.	5.25/1.012	5.25/1.012	5.2/1.011	5.25/1.014	5.5/1.016	5.5/1.019
	W. L. S.	6.5/1.023	5.25/1.014	6.2/1.011	5.25/1.014	5.25/1.014	5.3/1.017
	J. A. N.	6.75/1.008	7.0/1.013	5.25/1.028	5.25/1.023	5.57/1.024	5.75/1.024

*Blood pressure readings of W. L. S. were found to be as high as 200/135 several weeks before the study. He was confined to the hospital for fifteen days for observation before the study was begun.

All other chemical determinations on the blood serum and plasma were made by Mrs. T. Leake of the Central Laboratory of the University of California Hospital.

In each instance, the low sodium and potassium intake was continued for three days. During this time daily specimens of blood were obtained for determination of the sodium and potassium content of the serum when the individual had been fasting for fourteen hours. All urine excreted was collected in twenty-four-hour lots and was measured for amount, specific gravity, and pH (Table I). The twenty-four-hour output of sodium and potassium excreted in the urine was determined (Chart 2). The blood pressure and the body weight were likewise determined daily when the subject had been fasting (Table I). At

the end of the three-day period all the chemical determinations of the blood serum and plasma were repeated (Table II).

Three persons (J. F. W., W. L. S., and J. A. N.) were continued on the same diet and fluid intake but with the additional administration of 6 Gm. of potassium chloride in enteric coated tablets for two days longer.

Two other patients were studied in the same manner. The first was in the normal group and the second had a history of arterial hypertension of at least ten years' duration but had normal blood pressure readings during the study. The results obtained in these persons are not reported in this paper because of incomplete data. Such data as were obtained, however, in both persons were similar to those obtained in other normal persons.

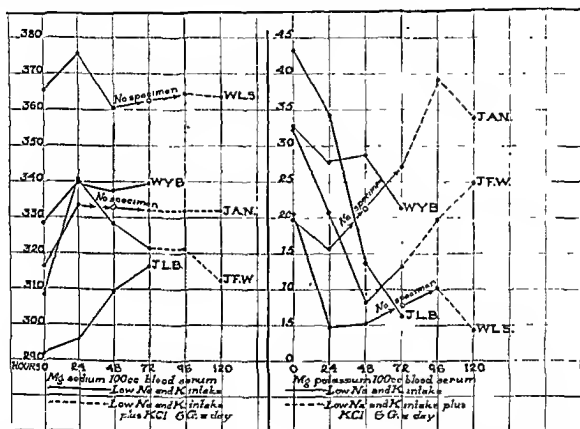


Chart 1—Blood serum level of sodium and potassium, normal and low sodium and potassium intake.

24 HOUR INTAKE		
	GM. NA	GM. K
W.L.S. Essential hypertension (early)	→ 0.0149	0.0039
W.Y.B. Control	→ 0.0604	0.0210
J.F.W. Essential hypertension (late)	→ Trace	0.0454
J.L.B. Essential hypertension (late)	→ Trace	0.1831
J.A.N. Control	→ 0.1220	0.04618

It was fully appreciated that the methods utilized in this study to restrict the intake of sodium and potassium were inadequate as far as normal diet requirements are concerned, and that the duration of the experiment was of a comparatively short period of time. However, it was felt that the reaction of the individual to salt deprivation might be tested in a more satisfactory manner by drastic restriction of both sodium and potassium for a shorter time than by permitting the person to have a less restricted intake of these two elements over a longer period of time. To prolong the drastic limitation of sodium and potassium intake would lead to a state of marked salt deficiency. It was felt that such a salt-deficient state would introduce faults in metabolism sufficient to render the interpretation of data obtained more difficult.

As mentioned above, no noticeable perspiration was present in any person during the experiment. No attempt, therefore, was made to measure or to

TABLE II
EFFECT OF LOW SODIUM AND POTASSIUM INTAKE ON BLOOD SERUM PROTEINS, BLOOD PLASMA CHLORIDES, NONPROTEIN NITROGEN, SUGAR, AND CARBON DIOXIDE

	J. L. B.		W. Y. B.		J. F. W.		W. L. S.		J. A. N.	
	NORMAL FASTING	72 HOURS LOW Na AND K FASTING	NORMAL FASTING	72 HOURS LOW Na AND K FASTING	NORMAL FASTING	72 HOURS LOW Na AND K FASTING	NORMAL FASTING	72 HOURS LOW Na AND K FASTING	NORMAL FASTING	72 HOURS LOW Na AND K FASTING
Blood serum proteins Total %	6.56	6.33	6.46	7.48	6.40	6.73	6.01	6.09	5.68	6.24
Albumin %	4.08	4.05	4.57	4.81	3.83	4.39	3.99	4.42	4.10	4.42
Globulin %	2.48	2.28	1.89	2.67	2.57	2.34	2.02	1.67	1.58	1.82
Albumin Globulin	1.65	1.78	2.42	1.80	1.49	1.89	1.98	2.64	2.60	2.42
Plasma chloride in mg./100 c.c. (as NaCl)	592	567	599	591	561	602	574	582	595	556
Plasma nonprotein nitrogen in mg./100 c.c.	31.6	40.0	32.2	33.0	29.7	36.4	31.6	38.0	27.5	35.3
Plasma sugar %	0.093	0.115			0.084	0.081	0.098	0.078	0.104	0.084
Plasma carbon dioxide in c.c.					55.5	48.2	47.7	50.2	54.7	46.0

analyze the fluid lost in the perspiration or to provoke excessive loss of fluid by artificial means, as has been reported by McCance.⁷

Tables I and II give data other than the sodium and potassium determinations. Charts 1 and 2 show in graphic form the fasting daily sodium and potassium blood serum levels and the daily excretion of sodium and potassium in the urine; they also give notation of the sodium and potassium intake in twenty-four hours.

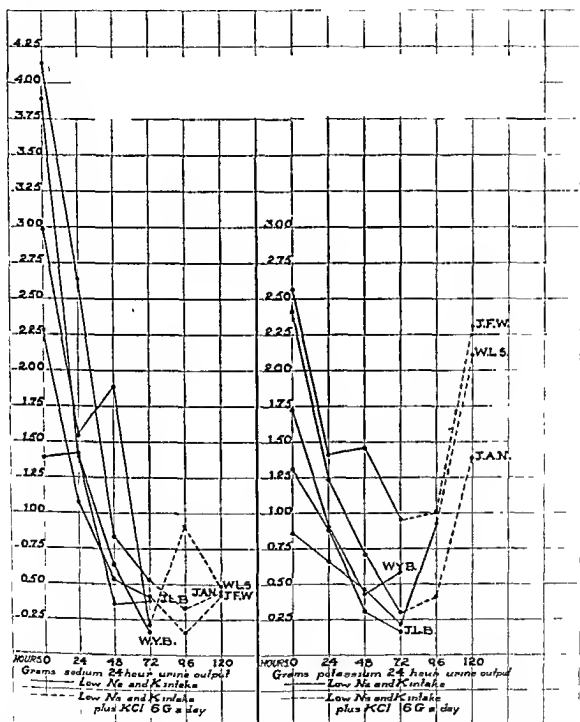


Chart 2.—Excretion of sodium and potassium in the urine, normal and low sodium and potassium intake.

DISCUSSION

Although the serum sodium level fluctuated in each individual when the sodium and potassium were restricted drastically, this change was of a minor degree in all five curves. When the two curves for the normal controls are compared with those for the patients with hypertension, no definite difference is seen between the levels of blood serum sodium in normal persons and the levels in persons with arterial hypertension.

The levels of serum potassium, however, fell following deprivation of sodium and potassium. It should be noted that in patients with definite arterial hypertension (J. L. B., J. F. W., and W. L. S.) a greater drop occurred than in two normal persons (W. Y. B. and J. A. N.). One normal individual (J. A. N.) at first had a moderate drop in the potassium level followed by a rise to above his normal level when sodium and potassium were restricted.

Chart 2 shows that the urinary output of both sodium and potassium decreased when these elements were restricted in the diet.

Two of the three patients (J. F. W. and W. L. S., both having arterial hypertension) on whom the experiment was continued for an additional two days with the addition of 6 Gm. of potassium chloride daily, failed to regain during this period their normal fasting levels of blood serum potassium. The change in the blood serum sodium in this group was of a minor degree. The addition of potassium chloride caused a definite increase in the excretion of potassium in the urine, with very little change in the amount of sodium excreted.

It was considered that the fall in blood pressure was probably due to several factors, namely, restriction of food and fluid intake as well as rest. Other data, such as fluid balance, body weight, and urinary pH shown in Table I, and serum protein, plasma chloride, sugar, and carbon dioxide combining power in Table II, showed only moderate variations and were considered to be of no great significance. It will be recalled that McCance⁷ demonstrated very marked changes in such blood constituents as serum proteins, urea, chlorides, and sodium in his patients in whom he produced a sodium chloride deficiency. None of the persons reported in this paper complained of the symptoms reported by McCance, such as aberrations of flavor, cramps, weakness, and lassitude. It is quite safe to conclude from our data in Tables I and II that our patients were not in a salt-deficiency state.

A rise was noted in the nonprotein nitrogen level of the blood plasma in all persons following sodium and potassium restriction.

It is well known that potassium is held largely in the intracellular fluid and sodium is held in the extracellular fluid. It was found in this study that the potassium level of the blood serum falls when the intake of both sodium and potassium is restricted. The significance of the greater decrease in the serum level of potassium in the patients with arterial hypertensive disease is not clearly understood at the present time.

SUMMARY AND CONCLUSIONS

In five male patients studied drastic restriction of sodium and potassium in the diet for a period of from three to five days produced no significant change in the blood serum level of sodium, but caused a drop in the blood serum level of potassium. This drop in the potassium level was of a greater magnitude in the three persons who had definite arterial hypertensive disease than in those who had normal blood pressure.

The urinary excretion of both sodium and potassium decreased with restriction of these two elements in the diet.

NOTE: Since the completion of the study here presented, my attention has been called to a paper by O. L. U. S. DeWesselow and W. A. R. Thomson entitled, "A Study of Some Serum Electrolytes in Hypertension," in the *Quart. J. Med.* 8: 361, 1939. These authors pointed out that in their experiments the serum of patients suffering from essential and malignant hypertension tended to show a lower level of potassium than that of patients with a normal blood pressure on the same diet.

REFERENCES

1. McQuarrie, Irvine, Johnson, R. M., and Ziegler, M. R.: Plasma Electrolyte Disturbance in Patient With Hypercorticoadrenal Syndrome Contrasted With That Found in Addison's Disease, *Endocrinology* 21: 762, 1937.
2. Anderson, Evelyn, Haymaker, Webb, and Joseph, Michael: Hormone and Electrolyte Studies of Patients With Hyperadrenocortical Syndrome (Cushing's Syndrome), *Endocrinology* 23: 398, 1938.
3. McCance, R. A.: Medical Problems in Mineral Metabolism (The Goulstonian Lectures, 1936), *Lancet* 1: 230, 704, 1936.
4. Butler, A. M., and Tuthill, Elizabeth: An Application of the Uranyl Zinc Acetate Method for Determination of Sodium in Biological Material, *J. Biol. Chem.* 93: 171, 1931.
5. Shohl, Alfred T., and Bennett, Helen B.: A MicroMethod for the Determination of Potassium as Iodoplatinate, *J. Biol. Chem.* 78: 643, 1928.
6. Strauss, Margaret B.: The Use of Thorium Nitrate in the Rapid Ashing of Serum and Urine. I. Adapted for Subsequent Potassium Determinations, *J. Biol. Chem.* 118: 331, 1937.
7. McCance, R. A.: Experimental Sodium Chloride Deficiency in Man, *Proc. Roy. Soc., London*, s. B. 119: 245, 1935.

THE EFFECT OF IRON ON PHOSPHORUS, CALCIUM, AND NITROGEN METABOLISM*

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THE administration of certain metals to experimental animals produces a low blood phosphorus and rickets. Both iron and aluminum,¹ when given in excess of the total phosphorus, were shown to precipitate the alimentary phosphorus as insoluble phosphates which were not absorbed and consequently caused a lowering of the blood phosphorus in guinea pigs. When ferrie chloride² was added to a nonrachitogenic diet, it rendered this same diet rachitogenic for rats and a low serum phosphorus resulted. It was found that iron and ammonium citrates exerted the same effect and reduced iron acted similarly. The rachitogenic effect could be prevented by adding excess phosphorus to the diet. Deobald and Elvehjem³ pointed out that the addition of a soluble iron salt to the diet in chicks produced severe rickets within five days and that the lowering of the blood phosphorus to this degree might affect other biologic processes as well as bone nutrition. The clinical implications from these animal experiments are obvious, and the possible harm resulting from the administration of large doses of iron during the period of growth and development of a child has been suggested. Brock and Diamond² state that large doses of iron continued over a

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TABLE I

CASE	DIAGNOSIS	AGE	SEX	BLOOD HEMO- GLOBIN GM. %	MIN- ERAL	CONTROL	PERIODS WITH IRON							
							I	II	III	IV	V	VI	VII	VIII
1	Anemia, hemorrhagic	45	F	7.80	Fe P Ca N	+ 0.11 + 0.24 - 0.06 + 0.43	+11.62 + 0.26 - 0.18 + 0.84	+ 13.73 + 0.28 - 0.19 + 1.13						
2	Anemia, hemorrhagic	43	F	7.02	Fe P Ca N	- 5.05 - 0.13 - 0.50 - 0.21	+14.06 + 0.37 - 0.11 + 1.60	+ 13.92 + 0.29 + 0.18 + 1.92						
3	Anemia, hemorrhagic	42	F	7.60	Fe P Ca N	- 2.96 ± 0.00 - 0.72 + 0.38	+12.20 + 1.74 - 1.18 + 3.96	+ 5.45 + 1.04 - 1.93 + 2.66						
4	Anemia, hemorrhagic	52	F	5.65	Fe P Ca N	- 2.14 + 0.22 - 0.31 + 0.90	+ 5.87 + 0.04 - 0.87 + 2.27	+ 16.03 + 0.20 - 0.01 + 1.24	+ 16.49 + 0.08 - 0.64 + 1.27	+ 12.25 + 0.06 - 0.75 + 0.42				
5	Anemia, idiopathic	44	F	7.45	Fe P Ca N	- 4.28 + 0.03 - 0.80 - 0.24	+53.25 + 0.29 + 0.68 + 0.42	+ 14.31 - 0.11 + 0.36 - 0.16	+ 65.40 - 0.01 + 0.01 - 1.79	+ 48.54 + 0.23 + 0.77 - 1.75				
6	Anemia, idiopathic	41	F	7.18	Fe P Ca N	- 6.66 + 0.27 - 0.28 - 1.34	+55.13 + 0.28 + 0.09 - 0.87							

Iron expressed as milligrams.
Phosphorus, calcium, and nitrogen expressed as grams.

7	Anemia, hemorrhagic	21	F	7.89	Fe	- 9.53 + 0.17 + 0.20 + 4.76	+71.04 - 0.03 - 0.04 + 5.78	+ 61.21 + 0.18 + 0.03 + 5.18	+114.40 + 0.10 + 0.23 + 4.97						
8	Anemia, hemorrhagic	23	F	5.97	Fe P Ca N	-12.70 + 0.25 - 0.08 - 0.05	+43.22 + 0.25 - 0.17 + 2.04								
9	Anemia, hemorrhagic	33	F	3.61	Fe P Ca N	-12.97 + 0.22 - 0.23 - 0.81	+43.14 - 0.16 - 0.23 + 4.64	+ 42.16 - 0.16 - 0.05 + 6.34							
10	Anemia, hemorrhagic	45	F	2.36	Fe P Ca N	+ 3.01 + 0.25 + 0.25 + 1.11	+83.74 + 0.44 + 0.31 + 1.28	+ 27.59 + 0.15 + 0.28 + 1.93	+ 36.62 - 0.26 - 0.05 + 1.33	+ 43.45 + 0.01 + 0.38 + 1.69	+ 93.54 + 0.03 - 0.11 + 1.01	-103.92 + 0.26 + 0.17 - 1.24	+66.14 + 0.24 - 0.16 - 0.37		
11	Lead poisoning	55	F	7.89	Fe P Ca N	+ 2.27 + 0.35 + 0.78 + 2.51	+153.22 + 0.29 + 0.62 + 2.48	+153.39 + 0.19 - 0.13 + 1.61	- 52.21 + 0.25 + 0.64 + 1.79	+ 19.56 + 0.20 + 0.77 + 0.70					
12	Anemia, idiopathic	34	F	5.60	Fe P Ca N	- 1.37 + 0.30 + 0.68 + 0.32	+309.67 + 0.48 - 0.00 + 2.27	+389.38 - 0.11 - 0.20 + 1.49							
13	Anemia, hemorrhagic	52	F	5.65	Fe P Ca N	- 2.14 + 0.22 - 0.05 + 0.90	+280.60 + 0.27 - 0.09 + 1.52	+ 83.97 - 0.31 - 0.94 + 0.87							
14	Anemia, idiopathic	41	F	4.80	Fe P Ca N	- 9.78 + 0.18 + 0.46 - 2.09	+282.61 - 0.04 - 0.37 - 0.12	+123.63 + 0.10 + 0.23 - 0.05	+283.53 + 0.09 + 0.42 + 0.05	+318.75 - 0.21 + 0.63 + 0.48	+330.42 - 0.13 + 0.64 + 0.56	+120.32 - 0.22 + 0.10 + 0.03	+97.06 + 0.31 + 0.81 - 1.21	-99.51 - 0.08 - 0.41 - 2.48	

TABLE I—CONT'D

CASE	DIAGNOSIS	AGE	SEX	BLOOD HEMO- GLOBIN GML. %	MIN- ERAL	CONTROL	PERIODS WITH IRON							
							I	II	III	IV	V	VI	VII	VIII
15	Anemia, hemorrhagic	42	F	7.60	Fe P Ca N	- 2.66 + 0.00 - 0.72 + 0.38	+233.63 + 0.19 - 0.47 - 0.29	+254.01 + 0.04 - 0.67 - 0.11						
16	Chronic arthritis	53	F	12.63	Fe P Ca N	+ 3.45 + 0.24 + 0.11 + 1.06	+203.39 + 0.13 + 0.11 + 0.65	+200.27 + 0.33 + 0.18 - 0.35	+342.78 + 0.22 + 0.04 - 0.28					
17	Chronic arthritis	41	F	11.99	Fe P Ca N	+ 2.64 + 0.33 + 0.61 + 0.71	+307.04 + 0.12 - 0.06 - 0.63	+333.77 - 0.18 + 0.62 + 1.51	+276.33 + 0.37 + 0.06 + 1.18					
18	Chronic arthritis	51	F	12.41	Fe P Ca N	- 1.63 + 0.13 + 0.05 + 0.12	+280.23 + 0.14 + 0.20 + 0.57	+288.62 + 0.09 + 0.36 - 0.68	+110.27 - 0.02 - 0.19 - 0.90	+220.65 + 0.23 + 0.54 - 0.49	+220.60 + 0.25 - 0.01 - 0.50			
19	Anemia, idiopathic	43	F	4.08	Fe P Ca N	- 2.90 - 0.07 + 0.02 - 0.69	+404.41 - 0.32 - 0.47 - 1.06	+304.47 - 0.30 + 0.25 - 0.64						

long period of time in childhood might have an effect similar to that observed in experimental animals, perhaps not by the actual production of rickets, but by evidences of malnutrition of the bones. Elvehjem⁴ also warns of the danger of administering large amounts of iron and advises that the dose be kept at a minimum. With these points in mind balance studies were carried out on 19 adults to ascertain the effect of iron administration on phosphorus, calcium, and nitrogen retention.

METHOD

The method of study was the same as that described in the first of a series of reports on iron balances.⁵ Sixteen patients with varying degrees of hypochromic anemia, either idiopathic in origin or secondary to hemorrhage, and 3 control patients without anemia were selected for the study. The control period was preceded by a three-day adjustment period on the balance diet before observations were begun. Each balance period was of six days' duration, and the results are reported as the average value per day. During the control period the iron intake was obtained from the diet alone. In subsequent periods iron and ammonium citrates were given in varying amounts. Nitrogen was determined by the Kjeldahl method, phosphorus by the method of Fiske and Subbarow,⁶ and iron by the method of Reis and Chakmakjian.⁷ Calcium determinations followed the method of McCradden.⁸

The diets contained 1 Gm. of protein per kilogram of body weight, and the dietary iron intake varied from 9 to 12 mg. a day. The phosphorus and calcium intake, derived exclusively from the food, was approximately 1.5 and 1.3 Gm. a day, respectively.

RESULTS

A summary of the iron, phosphorus, calcium, and nitrogen balances for each patient is given by periods in Table I. The age of the patients, who were all females, varied from 21 to 53 years. An examination of the average daily balances during the control periods shows no correlation between the storage of the substances and the age of the patient. All patients except the controls (Cases 16, 17, and 18) had hypochromic anemia of varying degree. As has been pointed out previously, there is no correlation between the degree of anemia and the iron retention⁹ either during the control period or with the administration of medicinal iron, and in this study the degree of anemia had no effect on the phosphorus, calcium, or nitrogen balances.

In Table II is given a summary of the balance studies showing the intake and balance for each of the four substances during both the control period and the period of iron administration. The results during the administration of iron are expressed as the average daily intake and balance for the period of observation, which varied from six to forty-eight days, rather than being given by periods. It will be seen that during the period of iron administration all patients retained this metal, the amount retained varying with the intake. Cases 1 to 4 received small amounts of iron intramuscularly, 1.5 grains of iron and ammonium citrates a day. Cases 5 to 11 received from 15 to 36 grains of iron and ammonium citrates orally a day, and Cases 12 to 19 received 45 grains of the same preparation by mouth.

TABLE II

CASE	IRON SUMMARY		PHOSPHORUS SUMMARY		CALCIUM SUMMARY		NITROGEN SUMMARY		DAYS	MEDICINAL IRON (GRAINS PER DAY)
	INGESTION	BALANCE	INGESTION	BALANCE	INGESTION	BALANCE	INGESTION	BALANCE		
1	11.89	+ 0.11	1.44	+0.24	1.25	-0.06	9.78	+0.43	6	0
	23.53	+ 12.68	1.45	+0.27	1.25	-0.18	9.79	+0.99	12	1.5
2	11.89	- 5.05	1.44	-0.13	1.25	-0.50	9.78	-0.21	6	0
	23.58	+ 13.99	1.50	+0.33	1.26	+0.04	10.41	+1.76	12	1.5
3	11.73	- 2.66	1.44	±0.00	1.26	-0.72	9.78	+0.38	12	0
	24.59	+ 8.87	1.45	+0.23	1.26	-0.26	9.84	+0.55	12	1.5
4	11.86	- 2.14	1.45	+0.21	1.26	-0.05	9.79	+0.90	6	0
	24.51	+ 12.66	1.45	+0.10	1.26	-0.57	9.82	+1.30	24	1.5
5	10.17	- 4.28	1.42	+0.03	1.27	-0.08	9.96	-0.24	6	0
	170.58	+ 46.72	1.42	+0.10	1.27	+0.45	9.97	-0.85	23	15
6	11.89	- 6.65	1.44	+0.27	1.25	-0.28	9.78	-1.34	6	0
	180.46	+ 55.13	1.43	-0.28	1.25	+0.09	9.85	-0.87	6	15
7	12.69	- 9.52	1.50	+0.17	1.28	+0.20	10.37	+1.76	6	0
	182.04	+ 82.22	1.55	+0.08	1.31	+0.07	11.04	+5.31	18	15
8	13.03	- 12.70	1.53	+0.25	1.29	-0.08	10.77	-0.05	6	0
	182.74	+ 43.22	1.62	+0.25	1.32	-0.17	11.85	+2.04	6	15
9	12.96	- 12.97	1.55	+0.22	1.31	+0.66	10.73	-0.81	6	0
	183.25	+ 42.65	1.72	-0.16	1.52	-0.12	12.49	+5.49	12	15
10	11.89	+ 3.01	1.44	+0.25	1.37	+0.24	9.78	+1.11	6	0
	267.31	+ 35.30	1.48	+0.12	1.37	+0.12	10.93	+0.80	42	36
11	11.49	+ 2.27	1.44	+0.35	1.34	+0.78	9.84	+2.51	6	0
	268.17	+ 68.48	1.49	+0.23	1.49	+0.48	11.01	+1.65	24	36
12	11.89	- 1.37	1.44	+0.30	1.34	+0.68	9.78	+0.32	6	0
	426.62	+299.52	1.44	+0.18	1.52	-0.10	10.93	+1.88	12	40
13	11.86	- 2.14	1.45	+0.21	1.26	-0.05	9.79	+0.90	6	0
	517.70	+182.29	1.45	-0.02	1.26	-0.52	10.11	+1.20	12	45
14	11.89	- 9.78	1.44	-0.18	1.25	+0.46	9.78	-2.09	6	0
	517.70	+182.11	1.44	-0.02	1.25	+0.26	10.08	-0.35	48	45
15	11.73	- 2.66	1.44	±0.00	1.26	-0.72	9.78	+0.38	12	0
	517.70	+243.82	1.44	+0.11	1.26	-0.57	10.14	-0.20	12	45
16	11.81	+ 3.45	1.45	+0.24	1.25	+0.11	9.87	+1.06	6	0
	517.47	+248.88	1.43	+0.23	1.24	+0.11	9.73	+0.01	18	45
17	11.78	+ 2.64	1.45	+0.33	1.27	+0.61	9.81	+0.71	6	0
	517.83	+305.71	1.46	+0.11	1.27	+0.21	10.16	+0.89	18	45
18	11.78	- 1.63	1.45	+0.13	1.27	+0.05	9.81	+0.12	6	0
	517.87	+224.08	1.46	+0.13	1.27	+0.18	10.17	-0.40	30	45
19	4.15	- 2.90	0.80	-0.07	0.71	+0.02	5.84	-0.69	12	0
	518.12	+354.44	1.04	-0.31	0.73	-0.11	9.54	-0.85	12	45

Iron expressed as milligrams.

Phosphorus, calcium and nitrogen expressed as grams.

Iron medication was iron and ammonium citrates.

The effects of iron administration on the retention of phosphorus, calcium, and nitrogen are best seen in Table III in which the three substances are considered separately.

Table III shows the change in the phosphorus balance in each patient by periods. In Column 1 is the state of balance during the control period ex-

TABLE III

CASE	PHOSPHORUS										CALCIUM										NITROGEN									
	PERIODS WITH IRON										PERIODS WITH IRON										PERIODS WITH IRON									
	CON- TROL	I	II	III	IV	V	VI	VII	VIII	CON- TROL	I	II	III	IV	V	VI	VII	VIII	CON- TROL	I	II	III	IV	V	VI	VII	VIII			
1	+	+	+							-	-	+							+	+	+									
2	-	+	+							-	-	+							-	+	+									
3	E	+	+							-	-	+							+	+	+									
4	+	+	-							-	-	+		+					+	+	+									
5	+	+	-							-	-	+	+						+	+	+									
6	+	+								-	-	+	+						+	+	+									
7	+	+	+							-	-	+	+						+	+	+									
8	+	+	-							-	-	+							+	+	+									
9	+	+	-							-	-	+							+	+	+									
10	+	+	-							-	-	+							+	+	+									
11	+	+	-							-	-	+							+	+	+									
12	+	+	-							-	-	+							+	+	+									
13	+	+	-							-	-	+							+	+	+									
14	+	+	+							-	-	+							+	+	+									
15	E	+	+							-	-	+							+	+	+									
16	+	+	+							-	-	+							+	+	+									
17	+	+	-							-	-	+							+	+	+									
18	+	+	-							-	-	+							+	+	+									
19	-	+	-							-	-	+							+	+	+									

Control indicates state of balance for each substance: positive, negative, or in equilibrium. Subsequent periods with iron administration indicate increased or decreased retention as compared to control period regardless of actual state of balance.

pressed as positive, negative, or in equilibrium. The other columns show the increase or decrease in phosphorus storage as compared to the control period, a + sign indicating that more was retained than during the control period and a - sign indicating that less was retained even though the patient might still have been in an actual positive balance. It is seen that in the first period of iron administration 8 patients retained less phosphorus than in the control period and 11 retained more. In the second period 10 of the 17 patients retained less than during the control period and 7 retained more.

In the first 4 patients, who received small doses of iron intramuscularly, there are ten observation periods during iron administration and in four of these, all in one patient, there was a decrease in phosphorus retention. In the other six periods there was an increase of phosphorus retention over the control period. It will be noted in Case 4 that less phosphorus was retained in each of the four periods on iron therapy than during the control period, but the patient was still in a positive balance for each period (Table I) as well as in a positive balance for the entire observation period of twenty-four days (Table II). If the explanation for the diminished phosphorus retention in experimental animals is correct (a precipitation of insoluble phosphates in the alimentary tract), there would be no reason to expect that intramuscularly administered iron would affect the phosphorus balance and there is no evidence of such an effect in these cases.

In the second group of 7 patients (Cases 5 to 11) the iron was administered by mouth in moderate amounts, from 15 to 36 grains of iron and ammonium citrates a day. There are twenty-two balance periods during iron administration in this group, and in sixteen of these there was a decrease in phosphorus retention. In only six periods was more phosphorus retained than during the control period. This might seem to indicate that decreased phosphorus absorption and retention occurred, but when the results of the third group of patients who received larger amounts of iron are considered, the significance of the preceding group is lost. In the third group (Cases 12 to 19) 45 grains of iron and ammonium citrates were administered daily by mouth. In the twenty-seven periods of observation during iron administration, there were twelve in which there was a decreased retention of phosphorus and fifteen in which the retention was increased. Since this larger dose of iron did not decrease phosphorus retention, it seems doubtful whether the diminished retention in the preceding group of 7 patients was due to iron ingestion. There were a total of forty-nine observation periods during orally administered iron, and in twenty-one of these there was increased phosphorus retention and in twenty-eight a decreased retention.

In the entire group of 19 persons there were fifty-nine observation periods during iron administration, and in thirty-two of these periods less phosphorus was retained than during the control period, while in twenty-seven periods there was an increased phosphorus retention. There were 5 patients in actual negative phosphorus balance during iron administration, whereas there were 3 in negative balance in the control period. These results

are interpreted as indicating that there is no significant change in the retention of phosphorus in the adult as a result of the administration of iron either orally or intramuscularly. It is our impression that the tendency toward decreased phosphorus retention is too slight and too inconsistent to be of significance.

In Table III are presented in similar manner the effects of iron administration on the retention of calcium. In thirty-two of the fifty-nine balance periods there was a diminished calcium retention as compared to the control period; in twenty-five instances the retention was increased, and in two periods there was no change. These figures are almost identical with those obtained for phosphorus, but a comparison of the tables shows that in individual cases and periods there is no correlation between the phosphorus and calcium retention. In the twenty-five periods of increased calcium retention there was increased phosphorus retention in only fourteen and a diminished phosphorus retention in eleven. Of the thirty-three periods with diminished calcium retention only twenty-one also showed diminished phosphorus retention. In the ten observation periods during intramuscular iron therapy in the first 4 patients there are five periods with increased, and 5 with decreased, calcium retention. In the second group of 7 persons who received iron by mouth there are twenty-two observation periods. In nine of these the calcium retention was increased and in thirteen it was decreased. There were twenty-seven balance periods during iron therapy in the last 8 patients (Cases 12-19) who received larger amounts of iron. There were eleven periods with increased, and 14 with decreased, calcium retention, and two periods with no change from the control period. During the control period there were 9 persons with calcium balance and 9 persons who were in an actual negative calcium balance for the entire period of iron administration. These results indicate that iron administration does not significantly affect calcium retention.

Table III also contains data on nitrogen metabolism. It is seen that the administration of iron did not affect nitrogen retention, and that there is no correlation between the retention of nitrogen and the retention of either calcium or phosphorus.

SUMMARY

The phosphorus, calcium, and nitrogen balances of 19 adult patients were determined during the administration of medicinal iron. No consistent or significant changes in the metabolism of these substances could be detected during the administration of iron either intramuscularly or orally.

REFERENCES

1. Cox, G. J., Dodds, M. L., Wigman, H. B., and Murphy, F. J.: The Effects of High Doses of Aluminum and Iron on Phosphorus Metabolism, *J. Biol. Chem.* 92: xi, 1931.
2. Brock, J. F., and Diamond, L. K.: Rickets in Rats by Iron Feeding, *J. Pediat.* 4: 442, 1934.
3. Deobald, H. J., and Elvehjem, C. A.: The Effect of Feeding High Amounts of Soluble Iron and Aluminum Salts, *Am. J. Physiol.* 111: 118, 1935.
4. Elvehjem, C. A., Siemers, A., and Mendenhall, D. R.: Effect of Iron and Copper Therapy on Hemoglobin Content of the Blood of Infants, *Am. J. Dis. Child.* 50: 28, 1935.

5. Fowler, W. M., and Barer, A. P.: Retention and Utilization of Orally Administered Iron, *Arch. Int. Med.* 59: 561, 1937.
6. Fiske, C. H., and Subbarow, Y.: The Colorimetric Determination of Phosphorus, *J. Biol. Chem.* 66: 375, 1925.
7. Reis, F., and Chakmakjian, H. H.: Colorimetric Method for Quantitative Determination of Iron in Blood in the Form of Dispersed Prussian Blue, *J. Biol. Chem.* 92: 55, 1931.
8. McCrudden, F. H.: The Determination of Calcium in the Presence of Magnesium and Phosphates: The Determination of Calcium in Urine, *J. Biol. Chem.* 10: 187, 1911.
9. Barer, A. P., and Fowler, W. M.: Influence of Gastric Acidity and Degree of Anemia on Iron Retention, *Arch. Int. Med.* 59: 785, 1937.

ALTERATION OF GLUCOSE TOLERANCE IN PATIENTS WITH DISEASE OF PITUITARY, THYROID, AND ADRENAL GLANDS BY CHANGES OF DIET*

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THE numerous reports of disturbance of carbohydrate metabolism in patients with dysfunction of the pituitary, thyroid, and adrenal glands, together with the development of this disturbance in experimental animals with altered function of these endocrine glands, appear to have definitely established a relationship between the function of these glands and carbohydrate metabolism. It has been shown also that alteration of the metabolic mixture through diet will produce changes in carbohydrate metabolism.¹ It is important in the proper treatment of patients and of academic interest to ascertain whether or not the alteration of such metabolism is due directly to dysfunction of these endocrine glands or to changes in the metabolic mixture. The present study was instituted to obtain data regarding this question. These data, which we recognize as incomplete, is submitted in hopes of arousing interest in the subject.

METHOD

Glucose tolerance curves were obtained in patients with acromegalia, pituitary dwarfism, hyperthyroidism, myxedema, Addison's disease, and paroxysmal hypertension due to a pheochromocytoma. The tolerance was ascertained in the morning after the patient had fasted for from twelve to fourteen hours. Blood samples were obtained from the finger tip before, one-half, one, one and one-half, two, and two and one-half hours after administration of 50 Gm. of glucose in aqueous solution orally. The metabolic mixture was then altered by change of diet for from three to twenty-four days and the glucose tolerance test was repeated. In all instances the tolerance curves were obtained after two types of diets, and in most cases after three. One diet was of a high carbohydrate and low fat content, another was of low carbohydrate and high fat content, and a third, of both the carbohydrate and fat content the same proportion as that in an

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average hospital diet. The caloric value of the diets varied from patient to patient, but were approximately the same for all diets prescribed for any one patient.

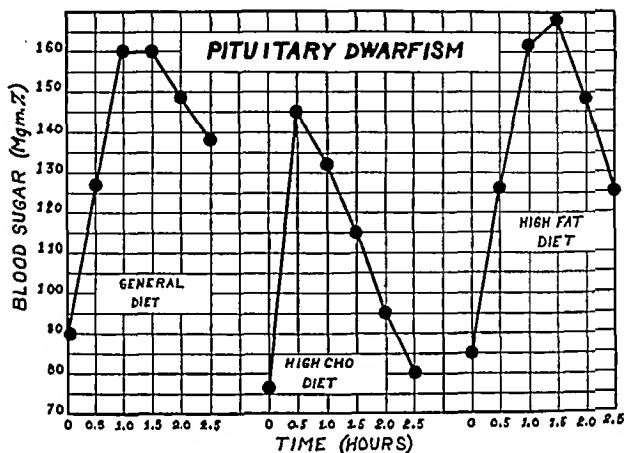


FIG. 1.

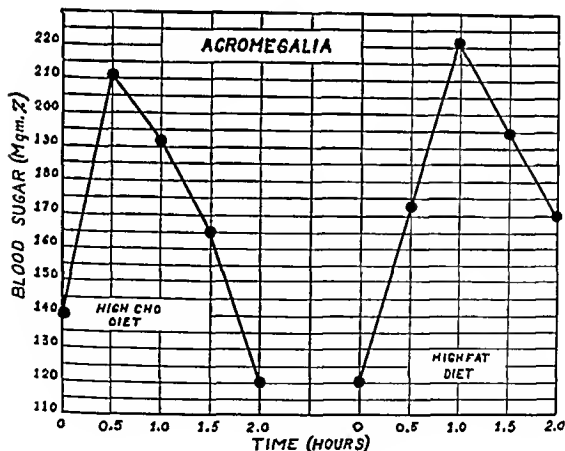


FIG. 2.

It was not deemed necessary to administer a given amount of glucose per kilogram of body weight for measure of the glucose tolerance because curves of one patient were not compared with those of another, but only with those of the same patient under different dietary conditions.

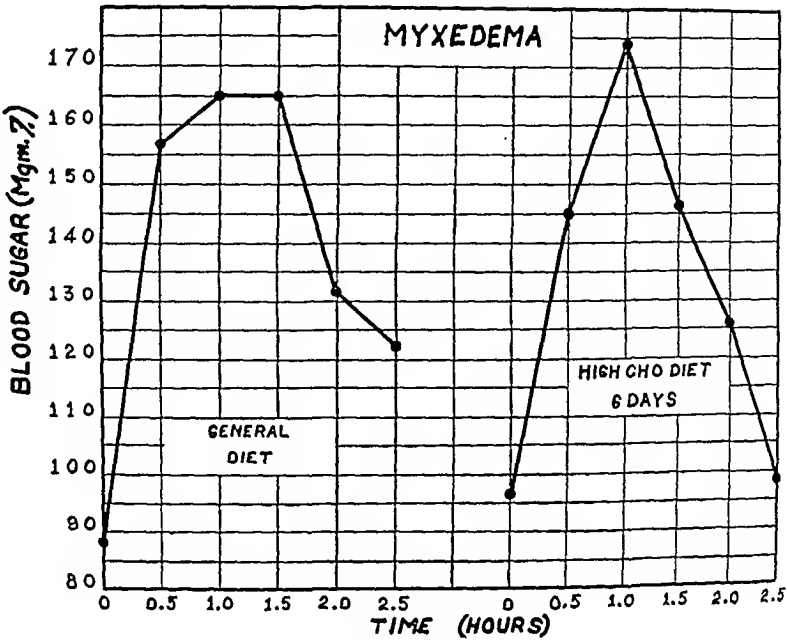


Fig. 3.

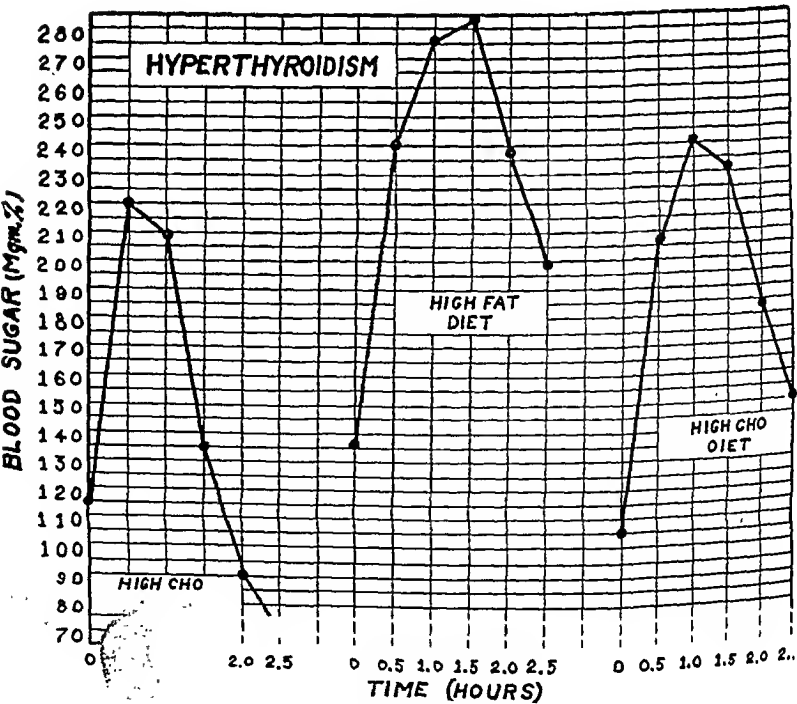


Fig. 4.

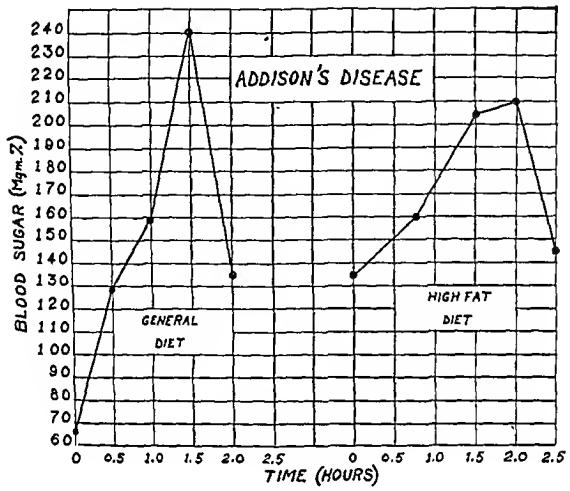


Fig. 5.

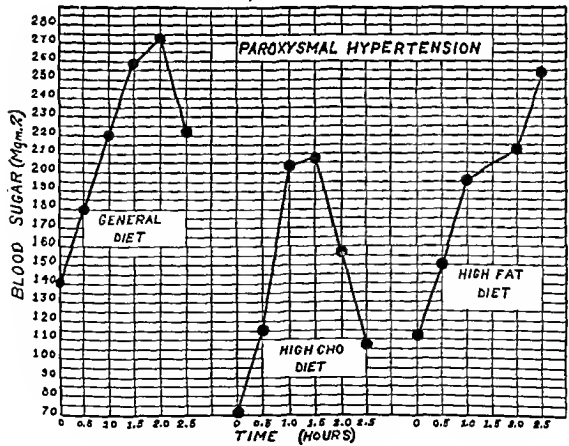


Fig. 6.

RESULTS

It is to be noted from Table I, which lists the number of patients with each disease and the effect of dietary alterations upon the glucose tolerance curve, that the tolerance was altered in all cases except in one of hyperthyroidism, and the results were questionable in one of Addison's disease. Representative alterations produced in the curve of each disease are shown in the accompanying graphs. Acetone bodies were not demonstrable in any of the seven patients in whom this point was ascertained during high fat, low carbohydrate diets.

TABLE I

NO. OF PATIENTS	DISEASE	EFFECT UPON GLUCOSE TOLERANCE	
		ALTERED	UNALTERED
2	Pituitary dwarfism	2	0
1	Acromegalia	1	0
2	Myxedema	2	0
8	Hyperthyroidism	7	1
1	Addison's disease	?	0
1	Pheochromocytoma	1	0
Total 15		13	1

COMMENT

It is recognized that there are too few cases of pituitary and adrenal dyscrasia in our study to permit any conclusions, but the results suggest that the disturbed carbohydrate metabolism may be due in part, if not entirely, to alteration of the metabolic mixture. If this is true, the disturbed carbohydrate metabolism can be corrected by proper dietary measures.

In regard to dysfunction of the thyroid gland, sufficient data are available to justify certain conclusions. There is usually a diminished glucose tolerance, but hypoglycemia may occur. Our results show that the diminished tolerance in hyperthyroidism is due to alteration of the metabolic mixture and that it can be remedied by a high carbohydrate and high caloric diet. The one patient who failed to show alteration of the tolerance curve had an initial normal curve and it was not altered by such a diet. The glucose tolerance can be diminished in these patients by a high fat and low carbohydrate diet. The one patient who failed to show this response developed hypoglycemia after two and one-half hours, and a high fat and low carbohydrate diet for eleven days thereafter did not alter the curve. A high carbohydrate and high caloric diet, on the other hand, was followed by a flattening of the curve and elimination of hypoglycemia. The explanation for this was not clear until we observed spontaneous hypoglycemia in patients due to a high fat diet.² It appears, therefore, that a high carbohydrate and high caloric diet is indicated in all cases of hyperthyroidism regardless of whether or not there is a diminished glucose tolerance or a hypoglycemia.

These observations show that the disturbed carbohydrate metabolism in hyperthyroidism is due to alteration of the metabolic mixture and is not due directly to dysfunction of the thyroid gland. The results show, in addition, that it is important to correct this altered glucose tolerance in such patients dur-

ing the preoperative treatment and that the maximum improvement should be one of the criteria which indicates whether or not the patient is prepared for thyroidectomy.

The patients with myxedema react to alterations of diet in the same manner as do normal persons, and this indicates that the diminished thyroid secretion is not a factor in the changes in carbohydrate metabolism in these cases.

SUMMARY

The glucose tolerance has been altered in patients with dysfunction of the pituitary, thyroid, and adrenal glands following alteration of the metabolic mixture by changing the carbohydrate and fat content of the diet. The tolerance was improved with a high carbohydrate diet and diminished with a high fat diet. It is suggested that in hyperthyroidism the maximum improvement in glucose tolerance be obtained before thyroidectomy is advised.

REFERENCES

1. Sweeney, J. S.: Dietary Factors That Influence the Dextrose Tolerance Test, *Arch. Int. Med.* 40: 818, 1927.
2. Swanson, L. W., and Greene, James A.: Further Observations on the Role of Diet in the Etiology and Treatment of Spontaneous Hypoglycemia, *J. LAB. & CLIN. MED.* To be published.

THE COMPARATIVE IODINE CONTENT OF WHOLE BLOOD AND SERUM*

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THE majority of patients with early, untreated exophthalmic goiter present an elevated blood iodine.¹ Veil and Sturm,² as early as 1925, demonstrated that there was an increase in the alcohol insoluble or "organic" iodine fraction in the blood of patients with hyperthyroidism. Later investigators have confirmed this observation.^{3, 4} This increased "organic" iodine *presumably* represents a greater circulation of the high iodine containing thyroid hormone or of its higher metabolic products.

The level of the "inorganic" fraction of iodine in the blood shows considerable variation.⁵ It is increased following the oral administration of inorganic iodine.³ A portion of the "inorganic" iodine represents the iodine of nutrition, i.e., iodine derived from the food, water, and, to a limited extent, air; and which enters the blood stream principally from the gastrointestinal tract, but also from the lungs. Normally this fraction should be small considering the relatively low daily intake of iodine.

We have observed that in patients on a low iodine intake analyses of the blood cells reveal only a trace of iodine, this being consistently increased by the

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oral administration of iodides. The present report deals with the results of a systematic investigation of the distribution of iodine in the serum and blood cells in patients without and with hyperthyroidism before and following the oral administration of iodine.

METHODS

The investigation was carried out on four groups of patients. The *first* group was composed of ten individuals without thyroid disease and the *second* of ten patients with exophthalmic goiter. These patients were maintained on the usual hospital diet which has a relatively low iodine content.⁶ They had not received iodine medication in any form for at least one month. The *third* group was made up of ten patients from the chest clinic, who had been lipiodolized for diagnostic purposes. No basal metabolic rate determinations were made on these patients; they showed no clinical evidence of thyroid disease. The *fourth* group was composed of ten patients with exophthalmic goiter who had received Lugol's solution or potassium iodide by mouth for from four to thirty days.

A sample of 70 c.c. of blood was drawn, with the patient in the fasting state. Twenty-five cubic centimeters of the blood were oxalated for the whole blood iodine determination. Forty cubic centimeters of blood were used to obtain from 15 to 25 c.c. of serum by clotting and centrifuging. Five cubic centimeters of blood were used for hematocrit determination. The analyses of the iodine in the whole blood and serum were made by the method of Matthews.⁷

TABLE I
IODINE CONTENT OF SERUM, BLOOD CELLS, AND WHOLE BLOOD

IODINE IN SERUM OF 100 C.C. OF WHOLE BLOOD (μ g)*	IODINE IN BLOOD CELLS OF 100 C.C. OF WHOLE BLOOD (μ g)	TOTAL IODINE IN SERUM PLUS CELLS OF 100 C.C. OF WHOLE BLOOD (μ g)	IODINE CONTENT OF 100 C.C. OF WHOLE BLOOD BY ANALYSIS (μ g)
4.2	0.2	4.4	4.2
5.0	0.4	5.4	5.2
35.0	2.3	37.3	38.1
924.0	203.0	1,127.0	1,058.0

*A microgram (μ g) equals 0.001 mg.

The percentage of iodine in the serum was obtained by the use of the following formula, in which I_b and I_s represent the concentration of iodine in the whole blood and serum, respectively, while the cell volume of the blood is expressed by V :

$$\text{Per cent of iodine in serum} = \frac{I_s (100 - V)}{I_b}$$

As a check on the accuracy of the analytical methods employed, the actual iodine content of the blood cells was determined in four patients. Following the removal of the serum by centrifuging, the blood cells were washed five times with normal saline and then analyzed by the usual method. Twenty cubic centimeters of packed cells were used. The results of this procedure are shown in Table I. The results obtained by analyses of the whole blood as compared to those by adding the separately determined iodine content of serum and blood cells show only a small deviation. The present method of iodine analysis is accurate to within 5 per cent for the amount of iodine ordinarily found in blood.

OBSERVATIONS

In the ten individuals without thyroid disease (Table II) the basal metabolic rate was within normal limits, averaging plus 5 per cent, with fluctuations of from minus 13 to plus 8. With a low iodine intake the whole blood iodine averaged 4.0 μg per cent, with variations of from 3.1 to 5.6 μg per cent. This is a normal blood iodine for this region by the present method of iodine analysis.⁷ The serum iodine concentration showed a similar variation. It ranged from 5.9 to 10.2, averaging 7.1 μg per cent. One hundred cubic centimeters of blood contained from 3.1 to 5.0 μg of serum iodine, with a mean value of 3.8 μg . The blood cells of 100 c.c. of whole blood contained only 0.2 μg of iodine, taking the average of all ten determinations. The serum iodine thus constituted, on the average, 95 per cent of the total iodine of the blood.

TABLE II
COMPARATIVE IODINE CONTENT OF WHOLE BLOOD AND SERUM
Individuals Without Thyroid Disease
No Iodine Medication

CASE NO.	DIAGNOSIS	B.M.R. %	BLOOD IODINE μg %	SERUM IODINE μg %	CELL VOLUME %	IODINE IN SERUM OF 100 C.C. OF WHOLE BLOOD (μg)	% IODINE IN SERUM
1	Obesity	+ 4	3.1	6.9	53	3.2	103
2	Normal male	+ 6	3.2	5.9	48	3.1	97
3	Psychoneurosis	- 3	3.3	5.9	46	3.2	96
4	Normal male	- 8	3.7	6.8	50	3.4	92
5	Ulcer of leg	+ 8	3.9	6.6	47	3.5	91
6	Normal male	- 1	4.0	7.0	47	3.7	93
7	Normal male	+ 5	4.0	7.4	43	4.2	105
8	Normal female	+ 6	4.2	7.2	43	4.1	98
9	Arthritis	+12	5.1	7.1	37	4.5	88
10	Normal female	-13	5.6	10.2	51	5.0	90
Averages		+ 5	4.0	7.1	46	3.8	95

The basal metabolic rate was elevated in the ten patients with exophthalmic goiter (Table III). The lowest basal rate was plus 39, the highest was plus 85 per cent, averaging plus 60 per cent. The whole blood iodine was increased in all ten patients, ranging from 5.5 to 15.7 μg per cent. The average of 10.0 μg per cent was two and a half times greater than the normal blood iodine. The serum iodine showed a similar elevation, fluctuating from 10.4 to 25.0 μg per cent in these patients with exophthalmic goiter. The average was 18.0 μg per cent as compared with 7.1 in the patients without thyroid disease. The serum of 100 c.c. of whole blood contained from 5.3 to 14.7 μg of iodine, averaging 9.6 μg . The blood cells of the same amount of whole blood contained only 0.4 μg . In these patients 95 per cent of the whole blood iodine was in the serum.

In the ten patients without thyroid disease, but who had been lipiodolized, the whole blood and serum iodine reached an unusually high level (Table IV). This increased blood iodine was due to the high absorption of the iodine of the lipiodol from the gastrointestinal tract. Fluoroscopy following lipiodolization revealed the presence of lipiodol in the stomach in all cases. The absorption from the bronchial tree is low, as has been shown in a previous report.⁸ The whole blood iodine ranged from 18.5 to 3153.0 μg per cent, with a serum iodine concentration of from 25.7 to 4232.0 μg per cent. The serum of 100 c.c. of whole

TABLE III
COMPARATIVE IODINE CONTENT OF WHOLE BLOOD AND SERUM
Patients With Exophthalmic Goiter
No Iodine Medication

CASE NO.	DURATION OF SYMPTOMS	B.M.R. %	WHOLE BLOOD IODINE $\mu\text{g } \%$	SERUM IODINE $\mu\text{g } \%$	CELL VOLUME %	IODINE IN SERUM OF 100 C.C. OF WHOLE BLOOD (μg)	% IODINE IN SERUM
1	2 years	+40	5.5	10.4	49	5.3	96
2	1 year	+56	6.1	10.1	42	5.9	97
3	6 months	+46	8.9	17.0	45	9.3	104
4	1 year	+63	8.9	15.4	41	9.0	100
5	1 year	+84	9.6	16.3	44	9.1	95
6	6 months	+39	10.8	17.6	44	9.8	90
7	6 months	+65	11.2	22.0	51	10.8	97
8	3 years	+56	11.4	20.7	53	9.8	86
9	1 year	+85	12.3	25.8	51	12.6	102
10	6 months	+64	15.7	25.0	43	14.7	90
Averages		+60	10.0	18.0	46	9.6	95

TABLE IV
COMPARATIVE IODINE CONTENT OF WHOLE BLOOD AND SERUM
Patients Without Thyroid Disease
Iodine Medication*

CASE NO.	DIAGNOSIS	WHOLE BLOOD IODINE $\mu\text{g } \%$	SERUM IODINE $\mu\text{g } \%$	CELL VOLUME %	IODINE IN SERUM OF 100 C.C. OF WHOLE BLOOD (μg)	% IODINE IN SERUM
1	Fracture of tibia	18.5	25.7	56	11.3	61
2	Chronic empyema	45.7	66.0	52	31.7	70
3	Pneumocyst of lung	72.0	124.8	53	58.6	81
4	Pulmonary monilliasis	474.0	521.0	47	276.0	60
5	Arteriosclerosis	1,058.0	1,777.0	48	924.0	88
6	Bronchiectasis	1,666.0	2,476.0	48	1,288.0	77
7	Bronchiectasis	1,672.0	2,518.0	49	1,284.0	77
8	Bronchiectasis	2,539.0	3,407.0	41	2,010.0	79
9	Obesity	2,467.0	3,724.0	45	2,048.0	82
10	Pulmonary tuberculosis	3,153.0	4,232.0	42	2,455.0	78
Averages				48		75

*Blood drawn from twelve to twenty-four hours following lipiodolization.

TABLE V
COMPARATIVE IODINE CONTENT OF WHOLE BLOOD AND SERUM
Patients With Exophthalmic Goiter
Iodine Medication

CASE NO.	IODINE MEDICATION	B.M.R. %	WHOLE BLOOD IODINE $\mu\text{g } \%$	SERUM IODINE $\mu\text{g } \%$	CELL VOLUME %	IODINE IN SERUM OF 100 C.C. OF WHOLE BLOOD (μg)	% IODINE IN SERUM
1	Lugol's 1 week	+60	15.2	20.2	40	12.2	80
2	Lugol's 4 days	+36	16.5	21.2	40	12.7	77
3	Lugol's 4 days	+49	17.3	22.0	36	14.1	80
4	100 mg. KI 5 days	+39	40.3	50.8	50	25.4	63
5	100 mg. KI 5 days	+ 8	41.5	53.7	46	29.0	70
6	100 mg. KI 9 days	+22	44.0	62.0	47	33.0	75
7	100 mg. KI 5 days	+38	63.5	74.5	43	42.5	67
8	100 mg. KI 5 days	+22	69.4	95.2	53	42.5	73
9	Lugol's 5 days	+38	106.0	162.0	50	50.5	76
10	100 mg. KI 30 days	+40	107.0	130.0	51	81.0	60
Averages		+35			46	63.7	72

blood contained from 11.3 to 2455.0 μg of iodine. There was an absolute, as well as a relative, increase in the corpuscular iodine, the cells of 100 c.e. of blood containing from 7.2 to 698.0 μg of iodine, or from 12 to 39 per cent of the total blood iodine.

Although the total iodine concentration in the patients with exophthalmic goiter who had received iodine in the form of Lugol's solution or potassium iodide was not as high as in these lipiodolized, the percentage of iodine in the serum to the total blood iodine was approximately the same (Table V). The basal metabolic rates were lower than in the patients with exophthalmic goiter not on iodine medication, and averaged plus 35 per cent, ranging from plus 8 to plus 60. The lowest whole blood iodine was 15.2 and the highest was 107.0 μg per cent. The iodine of the serum showed a corresponding increase, ranging from 20.2 to 130.0 μg per cent. The serum of 100 c.e. of whole blood contained from 12.2 to 63.7 μg , or on an average, only 72 per cent of the total blood iodine. The blood cells of the same amount of whole blood contained from 3.0 to 43.3 μg of iodine, as compared with 0.4 μg in the patients with exophthalmic goiter but not on iodine medication.

DISCUSSION

The increased whole blood iodine in the patients with untreated exophthalmic goiter as compared with the normal blood iodine of the individuals without thyroid disease was due to the higher iodine concentration of the serum, and not to an increased iodine content of the cells. With a low iodine intake the corpuscular iodine was approximately the same in both conditions. It constituted, on an average, only 5 per cent of the total blood iodine. The iodine concentration of the cells was thus similar to that of the cerebrospinal fluid of patients both with and without hyperthyroidism, as previously reported.⁹ The average cerebrospinal fluid iodine concentration was found to be 0.5 μg per cent, as compared with the average corpuscular iodine concentration of 0.6 μg per cent in the twenty patients on a low iodine intake.

The corpuscular iodine would appear to represent the diffusible iodine fraction of the blood, and, we may assume, also a part of the nutritional iodine of the blood. An increased intake of iodine is followed by an increase in the diffusible iodine fraction of the blood, as evident by the higher corpuscular iodine concentration. However, this corpuscular iodine represents only a part of the nutritional iodine, as there is also a concomitant rise in the serum iodine content. A portion of the ingested iodine is diffusible, while the remaining part is retained in the serum.

The actual amount of iodine ingested by these patients was not determined. No attempt has been made to demonstrate a quantitative relationship between the iodine intake and the nutritional iodine concentration of the serum and cells.

The determination of the whole blood and serum iodine is of some practical value in the differential diagnosis of hyperthyroidism in that it makes possible the demonstration of an increased amount of diffusible iodine in the blood. An elevated whole blood iodine may be due to the increased serum iodine of hyperthyroidism, but may also be caused by an elevated nutritional iodine, brought about by the ingestion of high iodine content foods or the administration of

iodine. The finding of a high corpuscular iodine concentration indicates that the patient under consideration has received iodine medication, and that this is responsible for the elevated whole blood iodine.

SUMMARY

In ten individuals without thyroid disease and maintained on a low iodine diet; with normal metabolic rates and normal whole blood iodine averaging 4.0 μg per cent, the average serum iodine was 7.1 μg per cent. The serum iodine constituted, on an average, 95 per cent of the total blood iodine.

In ten patients with exophthalmic goiter, with an average basal metabolic rate of plus 60 per cent, and maintained on a low iodine intake, the average blood iodine was 10.0 μg per cent, the average serum iodine was 18.0 μg per cent. On an average, 95 per cent of the iodine of the blood was in the serum.

Following lipiodolization of ten patients without thyroid disease, the whole blood iodine ranged from 18.5 to 3153.0 μg per cent, with the serum iodine fluctuating from 25.7 to 4,232.0. From 61 to 88 per cent, or on an average 75 per cent, of the total iodine of the blood was in the serum.

In ten patients with exophthalmic goiter, with an average basal metabolic rate of plus 35 per cent, while receiving Lugol's solution or potassium iodide by mouth, the blood iodine concentration fluctuated from 15.2 to 107.0 μg per cent, with the serum iodine concentration varying from 20.2 to 130.0. From 60 to 80 per cent, or at an average 72 per cent, of the iodine of the blood was in the serum.

CONCLUSIONS

In individuals without thyroid disease and with a normal blood iodine, and also in those patients with exophthalmic goiter with an elevated blood iodine while on a low iodine intake, an average of 95 per cent of the total iodine of the blood is in the serum.

The elevated blood iodine of patients with exophthalmic goiter is due to an increased amount of serum iodine.

Following the oral administration of varying amounts of iodine or lipiodolization, there is an increased corpuscular iodine concentration, with a relative fall in the serum iodine to an average of 74 per cent of the total iodine of the blood.

REFERENCES

1. Curtis, G. M., and Puppel, I. D.: The Iodine Metabolism in Exophthalmic Goiter, *Ann. Surg.* 108: 574, 1938.
2. Veil, W. H., and Sturm, A.: Beiträge zur Kenntnis des Jodstoffwechsels, *Deutsches Arch. f. klin. Med.* 147: 166, 1925.
3. Holst, J., Lunde, G., Closs, K., and Pedersen, O. C.: Ueber den inneren Jodstoffwechsel bei primären Thyreotoxikosen (Primär-Basedow), *Klin. Wchnschr.* 7: 2287, 1928.
4. Gutzeit, K., and Parade, G. W.: Blutjodstudien; die Blutjodfraktionen, *Ztschr. f. klin. Med.* 133: 513, 1938.
5. Elmer, A. W.: Iodine Metabolism and Thyroid Function, London, 1938, Oxford University Press, pp. 100; 377-378.
6. Cole, V. V., Curtis, G. M., and Bone, M. L.: The Iodine Content of Hospital Foods, *J. Am. Dietet. A.* 10: 200, 1934.
7. Matthews, N. L., Curtis, G. M., and Brode, W. R.: Determination of Iodine in Biological Materials, *Indust. & Engin. Chem., Anal. Ed.* 10: 612, 1938.
8. Cole, V. V., Dunn, R. H., and Curtis, G. M.: The Intrapulmonic Absorption of Iodine, *J. Pharmacol. & Exper. Therap.* 53: 327, 1935.
9. Klassen, K. P., Bierbaum, R. L., and Curtis, G. M.: The Comparative Iodine Content of Blood and Cerebrospinal Fluid, *J. LAB. & CLIN. MED.* 25: 383, 1940.

COMPARATIVE STUDIES ON THE ABSORPTION OF SULFANILAMIDE*

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RECENT studies² of a solution of sulfanilamide† demonstrated that in the guinea pig and rabbit higher blood-free sulfanilamide concentrations are obtained with the solution than when sulfanilamide is given in equivalent dosage in other forms. Furthermore, the sulfanilamide appears in the blood more quickly when given as the solution, and its rate of elimination is not accelerated. The solution employed was essentially a mixture containing in each fluidounce approximately 30 grains of sulfanilamide in glucose, glycerin, and sodium lactate.

The question has arisen as to whether the sulfanilamide in the solution would be absorbed as rapidly as an equivalent amount of drug in the dry form, because of a possible linkage with the glucose of the solution. It has been postulated, also, that in persons with partial or complete achlorhydria and rapid gastric emptying time the sulfanilamide, if bound to glucose in the solution, might not be absorbed as quickly or as completely as when administered in dry form.

To answer these questions studies were made, employing the solution of sulfanilamide in glucose, glycerin, and sodium lactate, and for comparison a brand of sulfanilamide tablets accepted by the Council on Pharmacy and Chemistry of the American Medical Association. The subjects were five males and one female between the ages of 21 and 49 in apparent good health, and one male, 49 years old, and one female, 51 years old, both of whom were afflicted with pernicious anemia.

Vitamin C determinations were made whenever possible for the purpose of trying to establish some correlation between vitamin C gain or loss and the sulfanilamide concentration. While these cases are not sufficient to draw any conclusions, there is enough evidence of such correlation to warrant further investigation.

Quantitative determination of blood sulfanilamide was made according to the method of Bratton and Marshall.¹ Since a comparison was being drawn between free sulfanilamide and a solution which might possibly contain the drug in a conjugated form, all values given are in terms of free sulfanilamide. Total sulfanilamide, that is, free plus conjugated, was not determined.

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†The solution of sulfanilamide known as "solution of sulfanilamide in glucose, glycerin, and sodium lactate," was furnished through the courtesy of Donley-Evans & Co., St. Louis.

PROCEDURE AND RESULTS

The first patient was a white male, aged 26 years, weighing 190 pounds. At the beginning of the study his red blood cell count was 4,140,000; hemoglobin, 13.8 Gm. (Newcomer method); white blood cell count, 9,600; nonprotein nitrogen, 40 mg. per cent; blood sugar, 92 mg. per cent; blood vitamin C, 0.15 mg. per cent. Fifteen cubic centimeters of the solution of sulfanilamide (equivalent to 15 grains of sulfanilamide) was administered every four hours. Sulfanilamide determinations were carried out on the blood at approximately 25° C. one-half hour before the drug was given and at four-hour intervals thereafter. The results are shown in Table I.

TABLE I
SOLUTION SULFANILAMIDE IN GLUCOSE, GLYCERIN, AND SODIUM LACTATE

TIME	DOSAGE	MILLIGRAMS PER CENT	
8 A.M.	15 gr.	0.0	Carbon dioxide combining power 63 volumes per cent
12 noon	15 gr.	0.7	
4 P.M.	15 gr.	1.1	
8 P.M.	15 gr.	1.4	
12 midnight	15 gr.	1.8	
4 A.M.	15 gr.	2.3	
8 A.M.	15 gr.	2.7	
12 noon	Discontinued	2.9	
4 P.M.		2.1	Carbon dioxide combining power 57 volumes per cent
8 P.M.		1.6	
12 midnight		1.1	
4 A.M.		0.8	
8 A.M.		0.6	
12 noon		Not done	
			Carbon dioxide combining power 61 volumes per cent

A period of one week was allowed to elapse between the conclusion of the observations in Table I and the beginning of the following study. Fifteen grains of sulfanilamide tablets were administered every four hours as previously. The results are given in Table II.

TABLE II
SULFANILAMIDE TABLETS

TIME	DOSAGE	MILLIGRAMS PER CENT	
8 A.M.	15 gr.	0.0	Carbon dioxide combining power 67 volumes per cent
12 noon	15 gr.	0.5	
4 P.M.	15 gr.	0.9	
8 P.M.	15 gr.	1.2	
12 midnight	15 gr.	1.5	
4 A.M.	15 gr.	1.9	
8 A.M.	15 gr.	2.2	
12 noon	Discontinued	2.4	
4 P.M.		2.0	Carbon dioxide combining power 54 volumes per cent
8 P.M.		1.6	
12 midnight		1.4	
4 A.M.		1.1	
8 A.M.		0.7	
12 noon		0.3	
			Carbon dioxide combining power 58 volumes per cent

The nonprotein nitrogen immediately following this study was 40 mg. per cent; the blood sugar, 87 mg. per cent. The blood picture showed no changes.

In this series of observations the same patient was used as in the first study, but after a period of two weeks. At this time, the nonprotein nitrogen was 42 mg. per cent, and the blood sugar was 87 mg. per cent. Blood vitamin C was 0.13 mg. per cent immediately before and 0.04 mg. per cent immediately following the experiment. Fifteen cubic centimeters of the solution of sulfanilamide was administered every four hours; sodium bicarbonate (approximately 20 to 30 gr. every two hours) was given throughout the test period in sufficient quantity to keep the urine alkaline during that time. Sulfanilamide determinations were made on the blood one-half hour before each dose of the drug was administered. The results are given in Table III.

TABLE III

SOLUTION SULFANILAMIDE IN GLUCOSE, GLYCERIN, AND SODIUM LACTATE,
AND SODIUM BICARBONATE

TIME	DOSAGE	MILLIGRAMS PER CENT	URINE	
8 A.M.	15 gr.	0.0	Alkaline	Carbon dioxide combining power 68 volumes per cent
12 noon	15 gr.	0.4	Alkaline	
4 P.M.	15 gr.	0.7	Alkaline	
8 P.M.	15 gr.	1.0	Alkaline	
12 midnight	15 gr.	1.7	Alkaline	Carbon dioxide combining power 84 volumes per cent
4 A.M.	15 gr.	2.1	Alkaline	
8 A.M.	15 gr.	2.4	Alkaline	
12 noon	Discontinued	2.9	Alkaline	Carbon dioxide combining power 86 volumes per cent

The second patient was a white male, aged 30 years, weighing 194 pounds. At the start of the experimental period his red blood cell count was 4,870,000; hemoglobin, 15.1 Gm. (Newcomer method); white blood cell count, 8,400; nonprotein nitrogen, 24 mg. per cent; blood sugar, 81 mg. per cent. A blood vitamin C determination was not done at this time, but several days later the blood vitamin C was 1.73 mg. per cent. Fifteen cubic centimeters of the solution of sulfanilamide (equivalent to 15 grains of drug) was administered every four hours. Blood sulfanilamide determinations were made at approximately 25° C. one-half hour before each dose of the solution was administered. The results obtained are shown in Table IV.

TABLE IV

SOLUTION SULFANILAMIDE IN GLUCOSE, GLYCERIN, AND SODIUM LACTATE

TIME	DOSAGE	MILLIGRAMS PER CENT	
8 A.M.	15 gr.	0.0	Carbon dioxide combining power 71 volumes per cent
12 noon	15 gr.	0.4	
4 P.M.	15 gr.	0.8	
8 P.M.	15 gr.	1.3	
12 midnight	15 gr.	1.7	
4 A.M.	15 gr.	2.1	
8 A.M.	15 gr.	2.6	
12 noon	Discontinued	2.8	Carbon dioxide combining power 69 volumes per cent
4 P.M.		2.4	
8 P.M.		2.0	
12 midnight		1.3	
4 A.M.		0.8	
8 A.M.		0.2	
12 noon		0.2	Carbon dioxide combining power 67 volumes per cent

A period of one week elapsed between the close of this and the following series of observations. The nonprotein nitrogen immediately preceding this series was 26 mg. per cent, and immediately following, 28 mg. per cent. Blood sugar determinations were not

made at this time. There were no significant hematologic changes. A dose of 15 grains of the brand of sulfanilamide tablets (5 gr.) previously employed was administered every four hours as shown in Table V.

TABLE V
SULFANILAMIDE TABLETS

TIME	DOSAGE	MILLIGRAMS PER CENT	
8 A.M.	15 gr.	0.0	Carbon dioxide combining power 68 volumes per cent
12 noon	15 gr.	0.3	
4 P.M.	15 gr.	0.7	
8 P.M.	15 gr.	1.1	
12 midnight	15 gr.	1.2	Carbon dioxide combining power 61 volumes per cent
4 A.M.	15 gr.	1.5	
8 A.M.	15 gr.	1.8	
12 noon	Discontinued	2.0	
4 P.M.		2.1	
8 P.M.		1.4	
12 midnight		1.1	
4 A.M.		0.3	
8 A.M.		0.1	
12 noon		Not done	
			Carbon dioxide combining power 58 volumes per cent

The third patient was a white male, 28 years old, weighing 181 pounds. His red blood cell count immediately preceding the observations was 4,070,000, with a Newcomer hemoglobin of 15.6 Gm.; the white blood cell count, 6,500; the nonprotein nitrogen, 29 mg. per cent; the blood sugar, 72 mg. per cent.

Neither the white blood cell count nor the hemogram showed any appreciable change subsequently. The blood vitamin C three days before the study was started was 1.47 mg. per cent as determined with a photoelectric adaptation of Ingall's method for blood vitamin C. Fifteen cubic centimeters of the solution of sulfanilamide (equivalent to 15 grains of drug) was administered every four hours. Sulfanilamide determinations at 25° C. were made on the blood one-half hour before each dose of the drug was administered. Concurrently, carbon dioxide combining power of the blood was determined as shown in Table VI.

TABLE VI
SOLUTION SULFANILAMIDE IN GLUCOSE, GLYCERIN, AND SODIUM LACTATE

TIME	DOSAGE	MILLIGRAMS PER CENT	
8 A.M.	15 gr.	0.0	Carbon dioxide combining power 76 volumes per cent
12 noon	15 gr.	0.2	
4 P.M.	15 gr.	0.7	
8 P.M.	15 gr.	1.3	
12 midnight	15 gr.	1.5	Carbon dioxide combining power 72 volumes per cent
4 A.M.	15 gr.	1.9	
8 A.M.	15 gr.	2.1	
12 noon	Discontinued	2.3	
4 P.M.		2.0	
8 P.M.		1.6	
12 midnight		1.1	
4 A.M.		0.4	
8 A.M.		0.2	
12 noon		Not done	
			Carbon dioxide combining power 67 volumes per cent Vitamin C determination 0.96 milligrams per cent

A period of one week was allowed to elapse between the foregoing and the following series. The nonprotein nitrogen immediately preceding was 27 mg. per cent, and immediately following, 28 mg. per cent. Neither blood sugar nor vitamin C determinations were done at this time. The hemogram and red and white blood cell counts were essentially the same as at the beginning of the series. Fifteen grains of the previously employed brand of sulfanilamide tablets were administered every four hours. As before, sulfanilamide determinations were made on the blood one-half hour before each dose of the drug was administered. Results are shown in Table VII.

TABLE VII
SULFANILAMIDE TABLETS

TIME	DOSAGE	MILLIGRAMS PER CENT	
8 A.M.	15 gr.	0.0	Carbon dioxide combining power 73 volumes per cent
12 noon	15 gr.	0.2	
4 P.M.	15 gr.	0.5	
8 P.M.	15 gr.	0.9	
12 midnight	15 gr.	1.3	
4 A.M.	15 gr.	1.6	
8 A.M.	15 gr.	1.9	
12 noon	Discontinued	2.1	Carbon dioxide combining power 67 volumes per cent
4 P.M.		2.0	
8 P.M.		0.8	
12 midnight		0.5	
4 A.M.		0.1	
8 A.M.		Trace	
12 noon		Not done	Carbon dioxide combining power 69 volumes per cent

A white male, aged 49 years, weighing 185 pounds, who for the past five years had been under treatment for pernicious anemia, was the subject in the fourth study. During the preceding eight months he had received no hydrochloric acid and only parenterally administered liver extract. Just before the experimental observations his red blood cell count was 4,010,000; Newcomer hemoglobin, 11.1 Gm.; white blood cell count, 7,300. The nonprotein nitrogen was 35 mg. per cent, and the blood sugar was 93 mg. per cent. Fifteen cubic centimeters of the solution of sulfanilamide were administered every four hours. Sulfanilamide determinations were made on the blood one-half hour before the drug was given. Immediately following the last sulfanilamide determination the stomach contents showed free nor combined hydrochloric acid. The results obtained are given in Table VIII.

TABLE VIII
SOLUTION SULFANILAMIDE IN GLUCOSE, GLYCERIN, AND SODIUM LACTATE

TIME	DOSAGE	MILLIGRAMS PER CENT	
8 A.M.	15 gr.	0.0	Carbon dioxide combining power 75 volumes per cent
12 noon	15 gr.	0.5	
4 P.M.	15 gr.	0.9	
8 P.M.	15 gr.	1.2	
10 P.M.*	Discontinued	1.9	Carbon dioxide combining power 69 volumes per cent

*Further observations were not possible because the patient found it necessary to leave the hospital.

The subject in the fifth study was a white female, 24 years old, weighing 105 pounds. At the start of this experiment she had a red blood cell count of 4,060,000, a hemoglobin of 11.6 Gm. (Newcomer method), and a white blood cell count of 6,200; her hemogram at

this time was essentially normal. Her nonprotein nitrogen, blood sugar, and urinalysis were well within the normal limits both before and immediately following the experiment. An arbitrary dose of 60 c.c. of the solution of sulfanilamide per day was administered in divided doses, 10 c.c. every four hours. Sulfanilamide determinations were done on the blood one-half hour before the drug was administered. All determinations were done immediately at approximately 25° C.

TABLE IX

*SOLUTION SULFANILAMIDE IN GLUCOSE, GLYCERIN, AND SODIUM LACTATE

TIME*	DOSAGE	MILLIGRAM PER CENT	
8 A.M.	10 gr.	0.0	Carbon dioxide combining power 73 volumes per cent
12 noon	10 gr.	0.4	
4 P.M.	10 gr.	0.7	
8 P.M.	10 gr.	1.2	
12 midnight	10 gr.	1.6	
4 A.M.†	10 gr.	Not done	
8 A.M.	10 gr.	3.1	
12 noon	Discontinued	3.2	Carbon dioxide combining power 67 volumes per cent
4 P.M.		3.4	
8 P.M.		2.7	
12 midnight		2.1	
4 A.M.†		Not done	
8 A.M.		1.1	
12 noon		0.8	Carbon dioxide combining power 69 volumes per cent
6 P.M.		0.4	

*These specimens were drawn at approximately the time indicated subject to thirty minutes' variations.

†Specimens were not drawn at these hours.

One week was allowed to elapse between the close of this series and the beginning of the next. A dose of 60 grains a day of a brand of sulfanilamide tablets (5 gr.) accepted by the Council was administered in divided doses as previously. Neither the blood chemistry nor the hematology at this time showed any significant variance from that immediately preceding the following experiment (Table X).

TABLE X

SULFANILAMIDE TABLETS

TIME*	DOSAGE	MILLIGRAM PER CENT	
8 A.M.	10 gr.	0.0	Carbon dioxide combining power 71 volumes per cent
12 noon	10 gr.	0.2	
4 P.M.	10 gr.	0.5	
8 P.M.	10 gr.	0.9	
12 midnight	10 gr.	1.3	
4 A.M.†	10 gr.	Not done	
8 A.M.	10 gr.	2.2	
12 noon	Discontinued	2.7	Carbon dioxide combining power 65 volumes per cent
4 P.M.		2.9	
8 P.M.		2.4	
12 midnight		1.8	
4 A.M.†		Not done	
8 A.M.		0.8	
12 noon		0.6	Carbon dioxide combining power 67 volumes per cent
6 P.M.		0.3	

*See footnote under Table IX.

†See footnote under Table IX.

In the sixth study the subject was a white male, 41 years old, weighing 138 pounds. At the start of this experiment he had a red blood cell count of 4,320,000, a hemoglobin of 14.3 Gm. (Newcomer method), and a white blood cell count of 9,100. His hemogram was normal, his nonprotein nitrogen was 27 mg. per cent, his blood sugar was 82 mg. per cent, and his vitamin C determination was 1.13 mg. per cent; a basal metabolism run three weeks prior to this test was plus 23. A dose of 60 c.c. of the solution of sulfanilamide a day was administered in divided doses, 10 c.c. every four hours. Determinations were done as previously.

TABLE XI
SOLUTION SULFANILAMIDE IN GLUCOSE, GLYCERIN, AND SODIUM LACTATE

TIME*	DOSAGE	MILLIGRAM PER CENT	
8 A.M.	10 gr.	0.0	Carbon dioxide combining power 76 volumes per cent
12 noon	10 gr.	0.3	
4 P.M.	10 gr.	0.7	
8 P.M.	10 gr.	1.4	
12 midnight	10 gr.	1.9	
4 A.M.†	10 gr.	Not done	
8 A.M.	10 gr.	2.9	
12 noon	Discontinued	3.4	Carbon dioxide combining power 67 volumes per cent
4 P.M.		3.1	
8 P.M.		1.8	
12 midnight		1.1	
4 A.M.†		Not done	
8 A.M.		Trace	
12 noon		Not done	Carbon dioxide combining power 69 volumes per cent

*See footnote under Table IX.

†See footnote under Table IX.

TABLE XII
SULFANILAMIDE TABLETS

TIME*	DOSAGE	MILLIGRAM PER CENT	
8 A.M.	10 gr.	0.0	Carbon dioxide combining power 69 volumes per cent
12 noon	10 gr.	0.2	
4 P.M.	10 gr.	0.5	
8 P.M.	10 gr.	0.9	
12 midnight	10 gr.	1.3	
4 A.M.†	10 gr.	Not done	
8 A.M.	10 gr.	2.0	
12 noon	Discontinued	2.3	Carbon dioxide combining power 58 volumes per cent
4 P.M.		1.8	
8 P.M.		1.1	
12 midnight		0.7	
4 A.M.†		Not done	
8 A.M.		0.3	
12 noon		Not done	Carbon dioxide combining power 61 volumes per cent

*See footnote under Table IX.

†See footnote under Table IX.

A period of one week was allowed to elapse between the close of this series and the beginning of the next study. A dose of 60 grains a day of a brand of sulfanilamide tablets (5 gr.) accepted by the Council was administered in divided doses as before. The blood chemistry and hematology of this subject showed no significant changes from those run before the experiment with the exception of the vitamin C determination, which was

0.72 mg. per cent. The individual in the seventh study was a 21-year-old white male weighing 149 pounds. His red blood cell count immediately preceding this experiment was 4,720,000, with a Newcomer hemoglobin of 15.8 Gm. His white blood cell count at this time was 7,600, and his hemogram was normal. His nonprotein nitrogen was 26 mg. per cent, his blood sugar was 92 mg. per cent, and his vitamin C determination was 1.26 mg. per cent. A dose of 60 c.c. of the solution of sulfanilamide a day was administered in divided doses. Sulfanilamide determinations were done on the blood one-half hour before each dose of the drug was administered. All determinations were done immediately and at approximately 25° C.

TABLE XIII

SOLUTION SULFANILAMIDE IN GLUCOSE, GLYCERIN, AND SODIUM LACTATE

TIME	DOSAGE	MILLIGRAM PER CENT	
8 A.M.	10 gr.	0.0	Carbon dioxide combining power 82 volumes per cent
12 noon	10 gr.	0.3	
4 P.M.	10 gr.	0.8	
8 P.M.	10 gr.	1.1	
12 midnight	10 gr.	1.3	
4 A.M.	10 gr.	Not done	
8 A.M.	10 gr.	2.5	
12 noon	Discontinued	2.6	Carbon dioxide combining power 78 volumes per cent
4 P.M.		2.0	
8 P.M.		1.4	
12 midnight		0.6	
4 A.M.		Not done	
8 A.M.		0.1	Heavy trace
12 noon		Not done	Carbon dioxide combining power 79 volumes per cent Vitamin C determination 0.62 milligrams per cent

A period of one week was allowed to elapse between the foregoing experiment and the following. Neither the blood chemistry nor the hematology showed any significant changes from that at the start of this experiment. A dose of 60 grains a day of a brand of sulfanilamide tablets (5 gr.) acceptable to the Council was administered in divided doses of 10 grains every four hours, as previously. Sulfanilamide determinations were done on the blood one-half hour before each dose of the drug was administered, with the exception of the 4 A.M. dose, at which time no blood was drawn for a determination.

TABLE XIV

SULFANILAMIDE TABLETS

TIME	DOSAGE	MILLIGRAM PER CENT	
8 A.M.	10 gr.	0.0	Carbon dioxide combining power 81 volumes per cent
12 noon	10 gr.	0.3	
4 P.M.	10 gr.	0.6	
8 P.M.	10 gr.	1.2	
12 midnight	10 gr.	1.4	
4 A.M.	10 gr.	Not done	
8 A.M.	10 gr.	1.9	
12 noon	Discontinued	2.2	Carbon dioxide combining power 72 volumes per cent
4 P.M.		1.7	
8 P.M.		0.9	
12 midnight		0.4	
4 A.M.		Not done	
8 A.M.		Very faint trace	
12 noon		Not done	Carbon dioxide combining power 79 volumes per cent

The blood chemistry showed no significant changes at this time over that preceding this experiment; the red blood cell count and hemoglobin were approximately the same; the white blood cell count was 4,800, with a normal hemogram.

A period of one week was allowed to elapse between this experiment and the following study. The patient's blood chemistry immediately preceding this experiment was essentially the same as previously. The white blood cell count was 9,200. Sixty cubic centimeters of sulfanilamide solution, with concomitant dosages of sodium bicarbonate, were administered as previously.

TABLE XV

SOLUTION SULFANILAMIDE IN GLUCOSE, GLYCERIN, AND SODIUM LACTATE
PLUS SODIUM BICARBONATE

TIME	DOSAGE	MILLIGRAM PER CENT	URINE
8 A.M.	10 gr.	0.0	Alkaline
12 noon	10 gr.	0.2	Alkaline
4 P.M.	10 gr.	0.6	Alkaline
8 P.M.	10 gr.	1.3	Alkaline
12 midnight	10 gr.	1.5	Alkaline

Tests were discontinued because of patient's refusal to cooperate further.

The individual in the eighth study was a white female, aged 51 years, weighing 109 pounds, who for nine years had been under treatment for pernicious anemia. At this time, her red blood cell count was 3,940,000, with a Neweomer hemoglobin of 12.2 Gm. Her white blood cell count was 6,600, with a normal hemogram. The nonprotein nitrogen and blood sugar values were well within the normal limits. A basal metabolism run five weeks prior to this test was plus 9. A modified G-I series showed a rather marked increase in stomach motility, and the gastric analysis showed a total achlorhydria.

An arbitrary dose of 60 c.c. of solution of sulfanilamide a day was administered in divided doses every four hours (10 c.c.). Sulfanilamide determinations were made on the blood one-half hour before the drug was administered. At the close of this experiment, a nasal tube was passed and the gastric analysis still showed a total lack of free and combined acid. At this time this test was discontinued because the patient felt dizzy and complained of a headache; the following day, although she refused further laboratory studies, she reported that these symptoms had all disappeared.

TABLE XVI

TIME	DOSAGE	MILLIGRAM PER CENT
8 A.M.	10 gr.	0.0
12 noon	10 gr.	0.4
4 P.M.	10 gr.	0.9
8 P.M.	10 gr.	1.5
10 P.M.		2.3

DISCUSSION

The results obtained in this study are summarized graphically in Table XVII. It will be seen that the maximum concentration of sulfanilamide attained in the blood in every case was higher when the drug was given in the form of the solution. With sulfanilamide tablets, excretion time was shorter, and the per cent loss of blood carbon dioxide combining power was greater.

In the face of a known, persistent, complete achlorhydria, a rise in blood sulfanilamide, comparable to that obtained in normal subjects, occurred when the drug was administered in the form of a solution.

When the solution of sulfanilamide was administered, together with large doses of sodium bicarbonate (20 to 30 gr. every two hours), no interference with absorption of the sulfanilamide occurred.

TABLE XVII

SUBJECT			TOTAL SULFANILAMIDE ADMINISTERED	MAXIMUM CONCENTRATION REACHED (MG.%)	TIME FOR EXCRETION (HOURS)	CO ₂ AT START (VOL. %)	CO ₂ AT MAXI- MUM CONEN- TRATION (VOL. %)	CO ₂ AT FINISH (VOL. %)	PERCENTAGE OF LOSS IN GLY- CEME FROM START TO MAXIMUM CONC.
1 Male 26 years 190 pounds	Solution of sulfanilamide		105	2.9	24	63	57	61	0.095
	Sulfanilamide tablets N.N.R.		105	2.4	24	67	54	58	0.194
	Solution of sulfanilamide and sodium bicarbonate	*	105	2.9		68	84	86	
2 Male 30 years 194 pounds	Solution of sulfanilamide		105	2.8	24	71	69	67	0.023
	Sulfanilamide tablets N.N.R.		105	2.0	20	68	61	58	0.103
3 Male 28 years 181 pounds	Solution of sulfanilamide		105	2.3	20	76	72	67	0.054
	Sulfanilamide tablets N.N.R.		105	2.1	16	73	67	69	0.052
4 Male 49 years 185 pounds	Solution of sulfanilamide	†	60	1.9		75	69		0.050
5 Female 24 years 105 pounds	Solution of sulfanilamide		70	3.4	24	73	67	69	0.052
	Sulfanilamide tablets N.N.R.		70	2.9	24	71	65	67	0.054
6 Male 41 years 138 pounds	Solution of sulfanilamide		70	3.4	20	76	67	69	0.118
	Sulfanilamide tablets N.N.R.		70	2.3	20	69	58	61	0.157
7 Male 21 years 149 pounds	Solution of sulfanilamide		70	2.6	20	82	78	79	0.045
	Sulfanilamide tablets N.N.R.		70	2.2	20	81	72	79	0.111
	Solution of sulfanilamide and sodium bicarbonate	*	50	1.5					
8 Female 51 years 109 pounds	Solution of sulfanilamide	‡	40	2.3					

*Twenty to 30 grains of sodium bicarbonate administered every two hours.

†Pernicious anemia, five years. Stomach contents showed neither free nor combined hydrochloric acid.

‡Pernicious anemia, nine years. Marked high motility. Gastric analysis showed total achlorhydria before and after this experiment.

CONCLUSIONS

In the human subject higher blood concentrations of free sulfanilamide are obtained when the drug is given as the solution than when administered in equivalent dosage in the form of tablets.

The rate of elimination of the sulfanilamide is no more rapid when given as the solution than when administered in other forms.

The loss of blood carbon dioxide combining power is less when the solution of sulfanilamide is administered than when the drug is given in tablet form.

Even in the face of a persistent, complete achlorhydria, a rise in blood concentration of sulfanilamide comparable to that seen in the normal subject is attained when the drug is administered in the form of the solution. Hence the influence of gastric hydrochloric acid is not required to permit absorption of the sulfanilamide into the blood stream. If a linkage between the glucose and sulfanilamide does exist, gastric acidity is not needed for its breakdown.

REFERENCES

1. Bratton, A. D., and Marshall, E. K.: A New Coupling Component for Sulfanilamide Determination, *J. Biol. Chem.* **128**: 537, 1939.
2. Siebert, W. J., and Loose, Frederiek: Observations on a Sulfanilamide Solution, *J. LAB. & CLIN. MED.* **25**: 1062, 1940.

LABORATORY METHODS

GENERAL

SIMULTANEOUS ARTERIAL AND VENOUS BLOOD CULTURES*

MAYNARD MURRAY, M.D., AND FRANK MOOSNICK, B.S.
CINCINNATI, OHIO

IN REVIEW of the literature on blood cultures one is impressed by the fact that a comparative study of the efficiency of arterial blood cultures as compared to venous is wanting. In cases of septicemia it is not uncommon to see petechial hemorrhages follow the distribution of a small artery. Because of these facts such a comparative study was conducted on 27 patients with clinical evidence of bacteriemia.

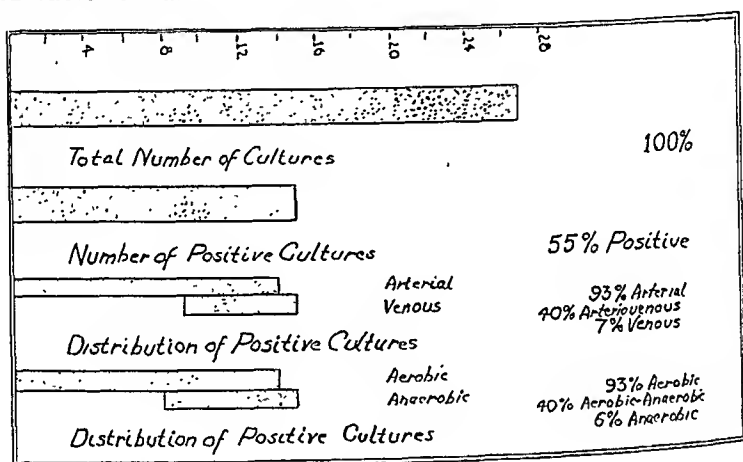


Fig. 1.

After surgical cleansing of the parts blood cultures were taken simultaneously from the cubital vein and from the femoral artery. The medium used was nutrient brain broth. The cultures were incubated at 37° C. for six days and subcultures were taken. The blood taken from each source was about 5 c.c. half of which was cultured aerobically and the other half anaerobically. As seen in Fig. 1 the results are as follows:

From 27 cultures taken from as many patients 15, or 55 per cent, were positive. In the 15 positive cases 14 were positive in the arterial culture, while only 6 were positive in the venous culture. One patient showed a positive venous culture and a negative arterial culture. As to the distribution of aerobic and anaerobic cultures, 15 aerobic and 7 anaerobic cultures were positive. One culture was positive in the anaerobic tube and negative in the aerobic tube.

From this experiment it is evident that arterial cultures are positive in a higher percentage of cases than are cultures taken from a vein.

*From the Laboratory of Physiology, University of Cincinnati and Longview State Hospital, Cincinnati.

A SIMPLIFIED APPARATUS FOR CONSTANT RATE INJECTIONS*

ALFRED E. KOEHLER, M.S., PH.D., M.D., SANTA BARBARA, CALIF.

IN STUDYING the ability of the animal body to utilize, store, or eliminate a substance injected intravenously, it is desirable to give it at a constant rate over a sufficiently long period of time, so that the organism can adapt itself and establish, if possible, an equilibrium. Similarly, in studying the effect of a substance such as a hormone upon the animal organism, the true response can usually better be evaluated if such a substance is given at a constant rate approaching physiologic or pathologic secretion.

Besides affording adequate time for diffusion and distribution equilibrium, continuous injection has the particular advantage over a single injection in that it frequently permits the observation of the change in equilibrium or response that may take place during the course of administration. Thus, if glucose is injected intravenously at a constant rate, the rapidity of disappearance of glucose from the blood stream constantly changes as the mechanism for sugar utilization becomes stimulated or, as in the case of the diabetic organism, first stimulated and then depressed. Similarly, if epinephrine is injected at a constant physiologic rate for two or four hours the blood pressure response may be quite different for the consecutive half hour intervals, in fact, may change from a pressor to a depressor effect. This difference of adaptation is often of primary importance and may be completely missed by a single injection even if prolonged to one-half hour.

APPARATUS FOR INJECTION

Several types of apparatus for constant rate injections that have been satisfactory for specific purposes have been described. One of the first motor driven pumps was described by Bock¹ in 1907, and modifications have been made by Friedman² (1910) and many others. Considerable improvement was, however, made in compactness, mechanical precision, and adaptability by Woodyatt³ in an apparatus described in 1917. This machine, as originally described, was subject to current fluctuations; due to the valvular mechanism involved, it never became popular for clinical use.

The apparatus to be described, although not capable of injection over indefinite periods as is the case with the Woodyatt pump, offers a simple, accurate means of constant rate injection, particularly practical for routine clinical tolerance tests. Our experience has shown that the average subject or patient cannot lie longer than two and one-half to three hours for injection without becoming unduly restless or uncomfortable, and consequently an apparatus for longer injection is usually not needed.

*From the Sansum Clinic and Santa Barbara Cottage Hospital, Santa Barbara.
Received for publication, November 29, 1939.

DESCRIPTION OF APPARATUS

Fig. 1 shows the front and side views of the apparatus. It consists essentially of a vertically supported syringe of 200 c.c. capacity or less that is actuated by a constant speed motor. The platform is about $7\frac{1}{2}$ inches square, and the two front corners support rigidly 2 steel tubes $\frac{5}{8}$ by 18 inches, $5\frac{1}{8}$ inches apart center to center. Sliding on these tubes are the stationary but adjustable support

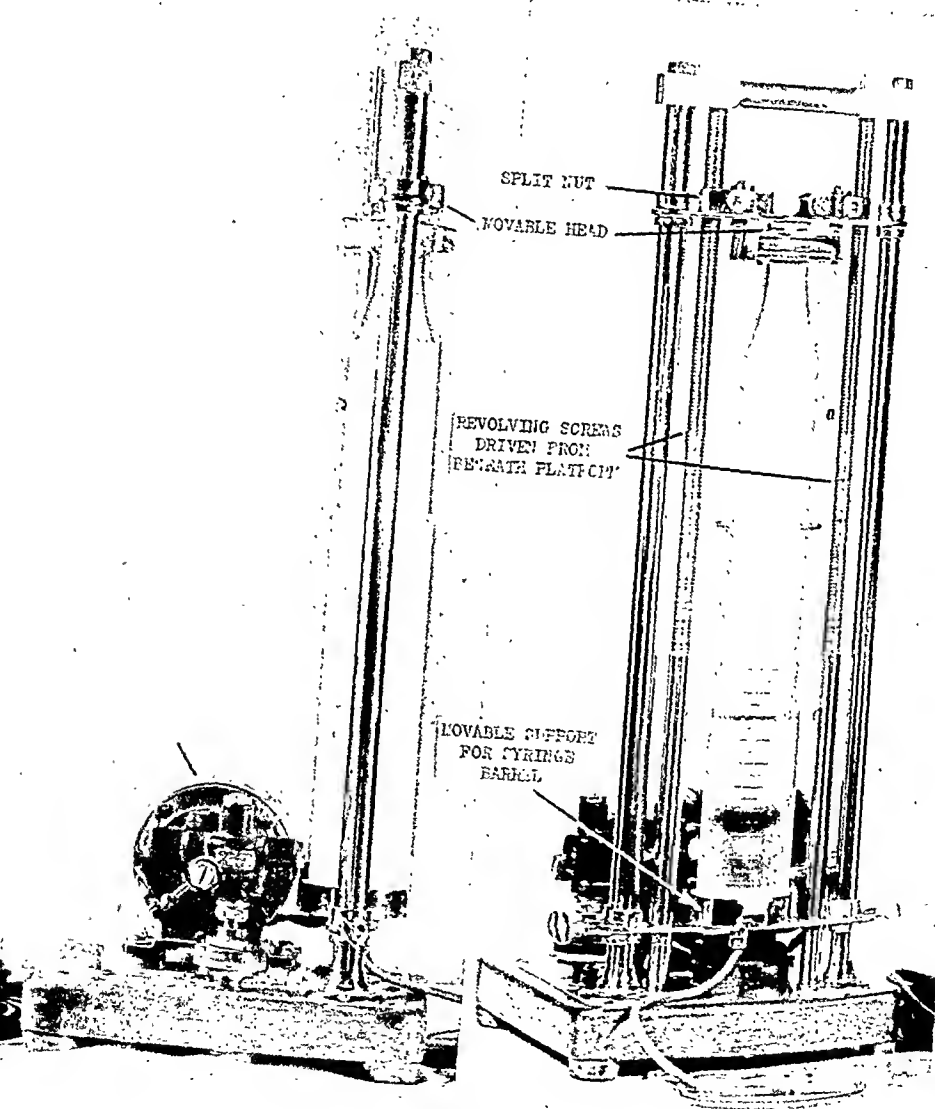


Fig. 1.

for the bottom of the barrel of the syringe and also the screw actuated head which clasps the top of the plunger. Inside of each stationary vertical support and parallel to it is a screw supported by a bearing in the top cross bar and also by a bearing in the base plate through which it extends. The screw shaft is $\frac{1}{2}$ inch diameter with 13 threads per inch. The head carrying the plunger

carries two split nuts which engage with the screws. Spring tension keeps the split nuts together to engage the thread, but pressure on the clip extensions with forefinger and thumb of each hand disengages the head so that it can be freely moved up or down on the outside supports.

The two screws are synchronously turned beneath the top plate of the base by sprockets and chain connected to the motor drive which extends through the base plate from above. The sprockets at the base of the screws are 2 inches in diameter with 20 teeth, and the sprocket on the motor drive extension shaft has 10 teeth.

A constant speed motor of 50 cycle 1,500 revolutions, $\frac{1}{10}$ horsepower, synchronous type with a 1:1,120 reduction drive extending downward is mounted on the back portion of the base.* Mounting the motor on a rubber base and inserting a rubber disk universal drive in the power extension minimizes vibration and noise.

The above type motor and reduction drive ratios result in the delivery of 100 c.c. per hour with the type of 200 c.c. syringe we have used.

An automatic shut-off switch operated when the syringe plunger is down is essential. The switch is mounted on the lower barrel support and is operated by a rod that extends up through an extension of the movable head. An adjustable collar fastened on the rod is engaged when the head has moved down sufficiently. The mounting of the switch on the lower movable support serves to maintain a constant shut-off relationship regardless of the position of the support.

Obviously, any rate of delivery can be obtained by using different drive sprocket ratios or by the introduction of various gears. Adjustable 3 or 4 speed transmissions can be built in below the base. On the other hand, we have found it desirable to make the driving mechanism as simple as possible so as to avoid mistakes.

To vary the amount of a substance to be injected it has in general proved more satisfactory to change the concentration of the solution rather than change the rate of injection of the solution. Thus glucose can be given at the rate of 10, 25, or 50 grams per hour by using a 10, 25, or 50 per cent solution injected at the rate of 100 c.c. per hour. Epinephrine or insulin can be diluted with normal saline, so that any desired amount can be given per hour.

The main advantage of this type of apparatus is its simplicity and practicability, so that it can be used for routine bedside work. The standard syringe and tubing can, of course, be easily sterilized.

REFERENCES

1. Bock, J.: Untersuchungen über die Nierenfunktion. I. Ueber die Ausscheidung der Alkalimetalle nach Injektion von Kaliumsalzen, *Arch. f. exper. Path. u. Pharmacol.* 57: 183, 1907.
2. Friedman, E.: Zur Technik der Durchströmung überlebender Organe, *Biochem. Ztschr.* 27: 87, 1910.
3. Woodyatt, R. T.: The Method of Timed Intravenous Injections, *J. Biol. Chem.* 29: 355, 1917.

*Such a constant speed motor may be obtained from the Bodine Electric Co., Chicago, Ill.

A SIMPLE PHOTOSTATIC CAMERA

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MORGANTOWN, W. VA.

PHOTOSTATIC records of books, journals, or other documents are a matter of great convenience and importance, especially where reprints are no longer available, or where library loans do not allow time for abstracting or for translation of foreign texts. "Bibliofilms" now provide a fairly convenient source of such records.

To meet daily desires, we have designed and built a photostatic camera of such simplicity and general utility that it seems suitable to present its details in such form that others may reproduce it. This device prints directly upon photographic paper, 5 by 7 inches, with only a slight reduction in size of text, in sharp white type upon a solid black ground. Even the finest types are sharply and legibly reproduced. Apart from the initial cost, prints may be made in quantity at approximately 2 cents each.

The apparatus consists of a baseboard with vertical grooved standards to support a camera, with its plate holder removed, on an adjustable slide. Fitted tightly above the camera is a light-proof horizontal box carrying a mirror at 45° angle, centered on the camera lens. The mirror is necessary to re-reverse the normally reversed image. Fitted into this box is a movable frame designed to receive an Eastman printing frame, 5 by 7 inches, fitting into a recess $\frac{3}{4}$ inch deep.

This movable frame is supported on a shelf attached to the camera slide, and is arranged to slide into or out of the mirror box, allowing $1\frac{1}{2}$ inches play for focusing. Guide strips and a wing-nut bolt to lock in focus are provided.

Three focusing movements are thus available: (1) the camera bellows; (2) the vertical movement of the camera slide; (3) the horizontal movement of the printing frame carrier. By proper manipulation of these movements all ordinary journal and book pages can be quickly reduced and sharply brought to focus on a 5 by 7 inch ground-glass screen.

The apparatus is easily constructed, chiefly of three-ply wood, conveniently available in large sheets. Bracing consists chiefly of 1 by 1 inch strips. Glue, wire brads, and $\frac{1}{8}$ inch wood screws are applied as indicated. The construction details are given in the illustrations and key to details. Basic dimensions alone are given.

We are using a Folmer and Schwing camera, 4 by 5 inches, as provided by Spencer Lens Co. (1924 catalog, No. 640, page 71). This is a speed graphic bellows camera with Kodak ball-bearing shutter and a 4 by 5 R. R. lens.

For illumination of books in photographing we use two Westinghouse par-38 projection flood bulbs, 150 watt, 120 volts. These are supported in clamps on metal standards at about 18 inches from the book, directed downward at

about 45° angle. With this illumination the exposure time runs around ten to twelve seconds, according to the selected printing paper.

From a variety of papers tested we have chosen Eastman Kodakline Bromide, single weight, smooth, semimatte, white paper. However, there is now available a new Kodabrom, grade A4, single-weight paper which promises even better contrast. We have not yet tried this new line, but from our experience with single-weight Kodabrom F4 paper we believe that this may be recommended.

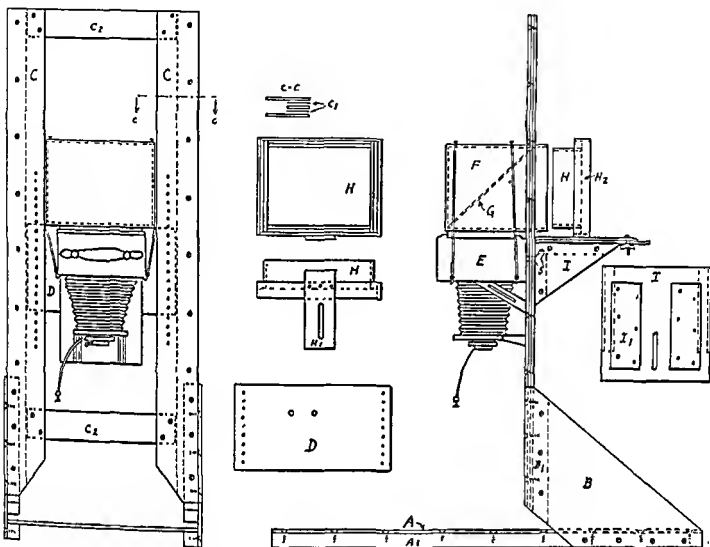


Fig. 1.—A, $\frac{1}{4}$ inch three-ply baseboard, 30 by 14 inches, supported on 1 by 1 by 30 inch runners (A1); B, $\frac{1}{4}$ inch three-ply gusset brace, $11\frac{1}{2}$ by $12\frac{1}{4}$ inches, supporting C-C through 1 by 1 by $3\frac{1}{2}$ inch strips (E1); C-C, $\frac{1}{4}$ inch three-ply support standards, 34 inches long, constructed of two $\frac{1}{4}$ by 3 by 34 inches and one $\frac{1}{4}$ by $1\frac{1}{2}$ by 34 inches, separated by two strips of cardboard (C1), to form groove for slide D; drilled with a series of $\frac{1}{8}$ inch holes for vertical adjustment of slide D; C2, two $\frac{1}{4}$ by 2 by $10\frac{1}{4}$ inch bracing strips to support C-C; D, $\frac{1}{4}$ by 6 by $10\frac{1}{4}$ inch slide for camera support; drilled with two $\frac{5}{16}$ inch holes to fit camera connection holes, and ends perforated by a series of $\frac{1}{8}$ inch holes to fit similar spacing on standards C-C. Slide D held in position at desired elevation by two 6-penny casing nails. Camera attached to slide D by knurled bolts provided with camera outfit: E, Spencer Photographic Camera (1924 catalog No. 640) supporting projection tunnel F, and clamped to it by four heavy rubber bands and wire hooks; bands looped over four brads on top of F; F, Projection tunnel, $\frac{1}{4}$ inch three-ply, 8 by 6 by 7 inches, holding G, silvered clear glass mirror, smooth and free of striations, $7\frac{1}{2}$ by $6\frac{1}{2}$ inches, set at exactly 45° angle, and held in place in F with small glued wood blocks; H, Focusing tunnel, fitting snugly into F, and movable on shelf I; H2, Collar built onto H, to hold standard 5 by 7 inch printing frame, and movable on shelf I to a depth of $\frac{3}{4}$ inch. Tunnel H allows $1\frac{1}{2}$ inch sliding insertion into F for focus. Tunnel H is guided in groove on shelf I by slide H1 ($\frac{1}{4}$ by 2 by $5\frac{1}{4}$ inches). Slide H1 has a 3 by $\frac{1}{4}$ inch slot to match slot on shelf I, fitted with $\frac{3}{16}$ by 1 inch bolt and wing-nut to lock H at proper focus; I, Shelf, $\frac{1}{4}$ by $7\frac{1}{2}$ by 8 inches, with two $\frac{1}{4}$ by 2 by 6 inch strips (I1) to form slide for H1; provided with 3 by $\frac{1}{4}$ inch slot to match H1.

Developing, Fixing, and Drying Details.—

1. Eastman developer, formula D-72 (about one minute).
2. Acid rinse bath (48 e.c. of 28 per cent acetic acid to 1 liter of water) (five to six seconds).

3. Eastman fixing bath, formula F1 (ten to fifteen minutes).

4. Wash at least thirty minutes in running water, or preferably until hypo test is negative (hypo test formula HT-1a). Note: Eastman chemicals are used throughout.

5. Prints are rolled and dried slowly on Ferrottype squeegee plates which are treated with "Peep-o-Day" squeegee paste (Karika Mfg. Co., Yale, Okla.), obtained through Eastman stores.

Printing suggestions.—Books or journals are laid flat on the baseboard A. The selected page is pressed flat under a clear sheet of window glass. The back of the book is bent out and held against a triangular rack (not illustrated) with sliding wire hooks to hold back the loose pages.

Printing frames are loaded and prints developed under the light of a Kodak safelight lamp, using a series OA Wratten safelight.

In printing a continuous series of pages we load and print six frames. With two persons working, one loads the frames, turns the pages, and manipulates the shutter; the other adjusts the focus, holds the printing frames in place, and operates the illumination switch. Working together, pages may be printed in a series at a rate of a little less than one per minute.

Complete reprint series are then conveniently bound with an Ace "pilot" stapler.

NOTE: Since the acceptance of this article for publication, we have found it more rapid to omit the use of plate holders. A glass plate is fixed in the collar (H2) and print paper is faced to the glass and held during printing by a felt-faced board. The printing speed is thus almost doubled.

AN OPEN RACK FOR THE SYRINGE OUTFIT WHEN IN USE

JOSEPH L. SZEKELY, M.D., FLUSHING, N. Y.

NEXT to the stethoscope, the syringe is probably the most frequently used medical instrument today. Yet the vast improvement that took place in the technical armamentarium during the last few decades has neglected to provide a commercially available, adequate solution to the simple, every-day problem of where to rest the sterile syringe outfit while in use. Before and after filling a syringe with the solution, and while it is being taken to the patient, the outfit should be placed in a secure position and the sterility of the needle and the withdrawn parts of the plunger should be safeguarded. This is somewhat difficult. The rolling tendency and the fragility of the syringe are a handicap. Various makeshift arrangements are resorted to, such as putting a piece of cotton on the tray under the tip of the syringe, placing the syringe with the needle extending over the edge of the table or tray, or even in an ash tray, etc. The disadvantages, such as the hazard of contamination, the bending of the needle point, and the possibility of breaking the syringe, are obvious and often happen.

The device described here answers this problem. It is a flat steel rack supported by two cylindrical legs with either one or three resting places for a

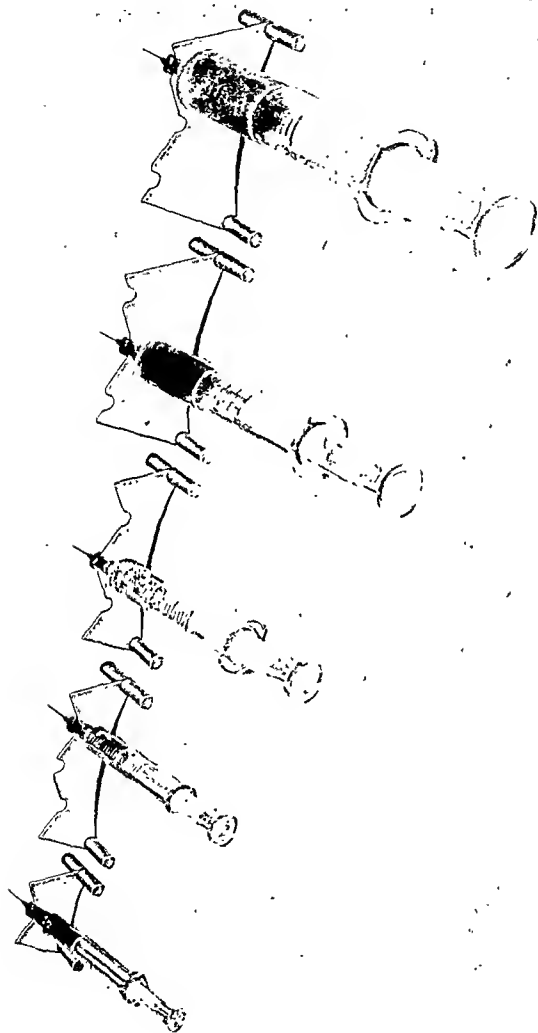


Fig. 1.

corresponding number of syringes. It is called the Steri-Rack and is made in three models. The small model will rest one syringe; the medium and the large, up to three syringes. The resting places have the shape of a semicircle that broadens out into a wide open V. The syringe outfit is usually placed in a slanting position with the hub of the needle or the tip of the syringe in the semicircle and the other end on the table or tray. The semicircle will just take the medium part of the hub of the needle. The front and rear prominences of the hub cannot pass through this opening; hence the syringe will not slip off the rack easily. If the needle happens to have a hub varying from the conventional style, the part of the syringe tip right behind the hub will rest in the semicircle with the same effect. Both glass and metal tipped syringes can be rested on the rack whether a needle is attached or not. The height of the resting levels varies to suit the different size syringes (Fig. 1).

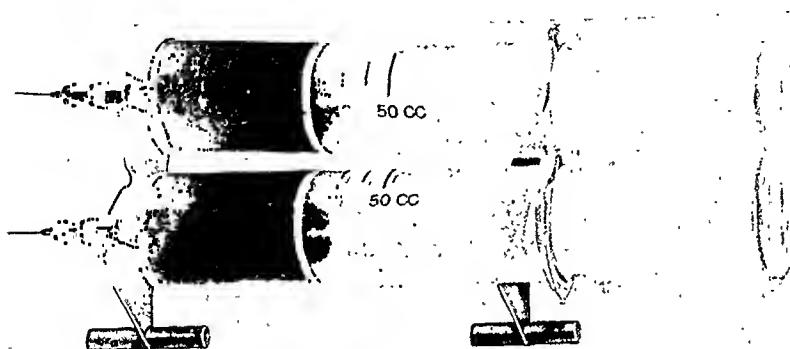


Fig. 2.

The V-shaped part of the resting place will take the barrel or the end of the plunger when a syringe is rested on two racks simultaneously (Fig. 2). The front end of the syringe outfit, either the hub or the barrel, rests on one rack, and the rear part, either the barrel or the end of the plunger, on another. Two different racks or two of the same model can be paired. The various heights of the semicircular and V-shaped resting places complement each other. This makes it possible to rest in a balanced position one or two of any size syringe, from the smallest up to a large metal ear syringe with both ends supported. Such an arrangement is advantageous, for instance, where prolonged sterility must be maintained or the barrel has a protruding side part, like that on a venoclysis syringe. The raised bottom line and the cylindrical legs, proportionate in length to the height and width of the rack, insure stability. The device is made throughout of stainless steel.

GONOCOCCUS COMPLEMENT FIXATION TEST

ALBERT M. DI GIOIA, A.B., SAN FRANCISCO, CALIF.

IN 1911, Schwartz and McNeal emphasized the necessity of using polyvalent antigens* in the gonococcus complement fixation tests because they believed the gonococcus belonged to a heterogeneous family. Until now, except for various research investigators, interest in the test has waned for the following reasons: first, the test would give no information in known acute or subacute cases; second, most laboratories were unable to obtain suitable antigens and positive controls; third, irregularities in the performance of the test. After a study of over 800 blood samples in this laboratory, we have attempted to eliminate some of the difficulties in the performance of the test. As a result, our experiences have been gratifying.

The following technique of the test is similar to that of Kolmer with certain modifications by this laboratory:

Solutions and Reagents.—

1. 0.85 per cent sodium chloride in distilled water.
2. 2 per cent sheep cells, defibrinated or citrated.
3. Anti-sheep hemolysin, the titer of which has been previously determined.
4. Complement—freshly pooled sera from three or more guinea pigs. Complement may prove to be one of the greatest sources of error in the test, thus making interpretations by the serologist difficult. Thomson, Park, and Hamann realized the instability of the complement and recommended the combined titration for both fixing and hemolytic powers. However, we found their procedure a somewhat laborious one. The hemolytic and fixability fractions can be assured by using the pooled sera of many guinea pigs, which when kept under optimum conditions of freezing or preservation and daily titration, will give good results.

In our laboratory pigs were bled once every week and the sera frozen in the refrigerator, 1 c.c. being placed in each of several test tubes. No complement after being thawed was returned for refreezing.

5. Patient's serum heated at 56° C. for fifteen minutes. Milky or hemolyzed sera are to be avoided.

As is recommended by Cohn, the inactivated serum is stored in the refrigerator overnight. Using freshly inactivated sera seems to give false serologic results due to increased lability of reactive substances.

Employing the use of sheep cells to remove native amboceptor is to be discouraged because of the number of anticomplementary reactions which are liable to occur. Too, large amounts of amboceptor are not easily removed by one absorption. The number of zone reactions which occur are so negligible that such technical complication is superfluous.

6. Gonococcus polyvalent antigen.

*Best polyvalent antigen available to us was donated by Parke, Davis & Co.
Received for publication, December 14, 1939.

Hemolytic titration: As bacterial antigens are rarely hemolytic, this titration may be completely omitted.

Anticomplementary titration: It is sufficient to titrate after the preparation of the antigen, and once every two months thereafter.

Antigenic titration: It is sufficient to perform this titration only once in every four to six weeks, using suitable positive control. In any case, the dose (2 units) employed in the test should be one-half to one-fourth the anticomplementary unit.

TABLE I

ANTIGEN TITRATION USING HEATED GONOCOCCUS-POSITIVE SERA

Antisera are not advisable as the antibodies vary so that the antigenic unit fluctuates according to the antiserum used. The antigenic unit is taken as the smallest amount of antigen giving complete inhibition of hemolysis. Two antigenic units are employed in the test.

ANTIGEN 0.5 C.C.	HEATED POSI- TIVE SERA 1:5 C.C.	COMPLEMENT 2 FULL UNITS C.C.	SENSITIZED SHEEP CELLS C.C.		
1:10	0.5	1	37° C. water	1	37° C. water Read
1:15	0.5	1	bath for 2	1	bath for one
1:20	0.5	1	hours	1	hour
1:25	0.5	1		1	
1:30	0.5	1		1	
1:35	0.5	1		1	
1:40	0.5	1		1	
1:45	0.5	1		1	
and higher up to 1:500					

TABLE II

COMPLEMENT FIXATION TEST

Readings are made as positive, doubtful, or negative. Positive is denoted by the inhibition of hemolysis in one or more dilutions of serum; doubtful, by slight inhibition of hemolysis in one tube only.

hemolysis in one tube only.								
TUBE	SERUM C.C.	SALINE C.C.	ANTIGEN 2 UNITS C.C.	COMPLE- MENT 2 FULL UNITS C.C.		SENSI- TIZED SHEEP CELLS C.C.		
1	0.3	0.2	0.5	1	6° to 8° C.	1	37° C.	Read when
2	0.2	0.3	0.5	1	refrigerator	1	water	controls
3	0.1	0.4	0.5	1	for 6 to 8	1	bath	have
4	0.05	0.45	0.5	1	hours	1	for	cleared
Serum	0.2	0.3	0.0	1		1	one	
control							hour	
Antigen	0.0	0.5	0.5	1		1		
control								
Positive	0.2	0.3	0.5	1		1		
control								
Negative	0.2	0.3	0.5	1		1		
control								

Complement Fixation Test.—No evaluation of the degree of positiveness was attempted, but since the gonococcus antibody is produced in most cases in small amounts, we have employed 0.3 c.c. of serum in the first tube, a larger amount than usual.

The sensitized sheep cells were prepared by mixing equal quantities of 2 per cent cells with two units of amboceptor and 1 c.c. of the mixture is added

to each tube both in the test proper and the antigenic titration. The reactions have been clearer-cut and faster.

We found that icebox incubation for six to eight hours was superior to fifteen to eighteen hours as it decreases the number of anticomplementary reactions and nonspecific positives. This may be explained by the fact that there is less destruction or absorption of complement in a shorter incubation period.

Readings of the test are made when antigen, serum, and negative controls have all cleared. This usually takes from ten minutes to twenty minutes. In this way weakly positive tests are not overlooked.

REFERENCES

- Frankman, W.: Significance of Complement Fixation Test in Gonorrhea, *New England J. Med.* 206: 11, 1932.
 Swan, C. S.: Results and Interpretation of the Complement Fixation Test for Gonorrhea, *New England J. Med.* 207: 601, 1932.
 Thomson, Annis E., Hamann, Agnes C., and Park, W. H.: Irregularities in the Complement Fixation Test for Gonorrhea, *J. Immunology* 29: 249, 1935.
 Cohn, Alfred: The Gonococcus Complement Fixation Test, *J. LAB. & CLIN. MED.* 22: 627, 1937.
 Kolmer, John A.: Serum Diagnosis by Complement Fixation, Philadelphia, 1928, Lea & Febiger.
 Kolmer, John A.: Infection Immunity and Biologic Therapy, Philadelphia, 1925, W. B. Saunders Co.
 Eagle, Harry: The Laboratory Diagnosis of Syphilis, St. Louis, 1937, The C. V. Mosby Co.
 Report of the Committee for Survey of Research on the Gonococcus and Gonococcal Infection, United States Government Printing Office, 1939.

HEMATOCRIT TUBE CLEANER*

ARTHUR C. STIRLING, M.D., ROCHESTER, N. Y.

THE cleaning and drying of fine bore hematocrit tubes of the Wintrobe type have been for some years a problem to the laboratory technician, intern, or medical student. Since the use of the hematocrit tube for blood sedimentation and erythrocyte volume determinations is constantly extending, especially in the study of arthritis and in following the progress of tuberculosis and other infections, there seemed to be merit in shortening at least one step in the process. The various methods employed to date have been time-consuming, bothersome and, if the blood is caked from long sitting, sometimes inadequate. The apparatus here described shortens the cleaning process to thirty seconds and eliminates the tediousness of other methods. The cleaner is conveniently used with equipment found in practically all hospital laboratories.

The cleaner consists of a chromium plated metal tube designed as illustrated in Fig. 1. The short arm is beveled and fits into the end of a rubber tube attached to a suction system; the same apparatus employed in cleaning the ordinary hemocytometer pipette may be used. The long arm, made of smaller bore stock, is long enough to extend nearly to the bottom of a Wintrobe hematocrit tube. In position for use this cleaner arm fits loosely inside the tube.

*From the University of Rochester School of Medicine and Dentistry.
 Received for publication, December 11, 1939.

Suction is applied at the beveled end which is connected to the rubber tubing. The hematocrit tube to be cleaned is slowly lowered over the long arm; the serum and packed blood cells are thus in great part removed. The cleaner with the hematocrit tube still in place is lowered into a wide-necked bottle containing water (Fig. 2). Water is sucked upward around the arm of the cleaner into the hematocrit tube and finally through the cleaner to the waste reservoir; the stream of water thus quickly removes clots of blood remaining in the tube. Agitation of the water, accomplished by repeatedly raising the tube above the surface of the water permitting bubbles of air to enter, increases the speed of the process.

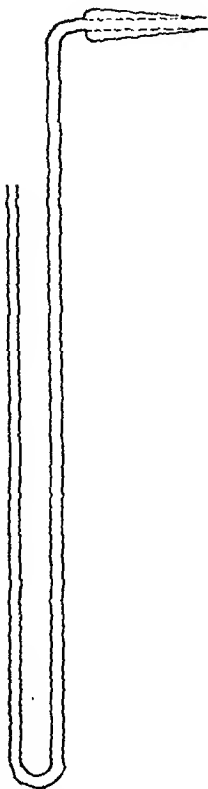


Fig. 1.

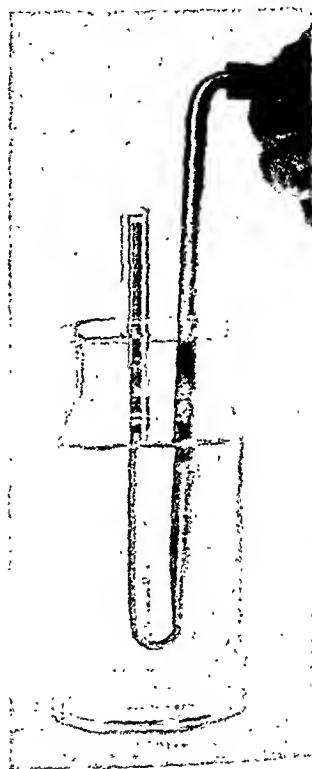


Fig. 2.

Fig. 1.—Hematocrit tube cleaner showing beveled short arm for connection with suction system.

Fig. 2.—Hematocrit tube cleaner with Wintrobe tube in place showing method of use with cleaning fluids.

Following the use of water the cleaner and tube are lowered in a similar manner, first into a bottle of alcohol which removes the excess water, then into ether which removes the alcohol. Only small amounts of alcohol and ether are needed if ample water is used. When the hematocrit tube is slowly removed from the cleaner, it is thoroughly dried upon evaporation of the ether by the air sucked into it.

Grateful acknowledgment is expressed to Mr. Raymond J. Bott of the Will Corporation, Dr. John S. Lawrence, Dr. Doran J. Stephens, and numerous others who have aided substantially in the development of this instrument.

The style of cleaner shown in Fig. 1 is available from the Will Corporation, Rochester, N. Y.

CHEMICAL

A METHOD OF COLLECTING AND TRANSPORTING SMALL BLOOD SAMPLES FOR HEMOGLOBIN DETERMINATION*

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NEW YORK, N. Y.

THE following procedure was evolved when it became desirable to determine the hemoglobin content of the blood of certain applicants for life insurance living at a distance from the laboratory. It allows the collection and transportation of small blood samples in a suitable condition for the determination of the hemoglobin content. The method is published in the belief that others may find it useful.

Blood is collected by capillary attraction from a finger-tip puncture into a roughly calibrated glass tube from which it is transferred into an accurately graduated pipette on being received in the laboratory. The tubes are lined with a small amount of hemolytic reagent and anticoagulant which are intimately mixed with the blood just after collection by tilting the tube and allowing the blood to flow from one end to the other. The tube is then sealed at both ends with plasticine and is sent to the laboratory in a kit designed for this purpose.

The kit consists of a cardboard carton containing two cellulose acetate tubes with metallic screw caps. One tube holds a spring lancet for making the finger-tip puncture, the other carries three capillary tubes, one of which is sealed with plasticine to show how this is done, the other two are prepared for the blood collection. Only one tube is needed but an extra one is included in case of mishap to the first. The plasticine is packed into a screw cap like those used to close the cellulose acetate tubes and is inserted in a glassine envelope for packing. To seal the blood tube after completing the collection, the ends are pressed through the plasticine against the metal cap several times until about $\frac{1}{4}$ inch of the bore is compactly filled. A bundle of slides, each in a glassine envelope, is bound with a rubber band and included in the outfit if an examination of the blood film is desired. Directions for taking the blood sample are printed on a folder which is wrapped around the two container tubes when packing them in the mailing carton. The outfit is sent as first class mail and requires only 12 cents postage. Similar kits are used for blood samples taken during sugar tolerance tests and for the Kline serologic test.

The capillary collection tubes are made from the smallest bore regular glass tubing available. The tubing has an outside diameter of 3 mm. and a bore of approximately 1.4 mm. It is cut into lengths of $5\frac{3}{4}$ inches, and the ends are

*From the Biochemical Laboratory of the Metropolitan Life Insurance Co., New York.
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smoothed down by rubbing on emery cloth or a similar abrasive. The tubes are marked with laboratory ink 4 inches from one end, the point to which blood is collected. On receipt in the laboratory the plugged ends of the tube are filed and broken off, and the blood is transferred from the tube to a 0.1 c.c. Folin capillary pipette by placing their tips together and allowing the blood to flow into the pipette.

Potassium oxalate is used as an anticoagulant and saponin to hemolyze the blood cells. The blood must be hemolyzed when it is freshly drawn, otherwise the cells are likely to settle along the bore of the tube or at one end and cannot afterwards be as thoroughly mixed with the plasma as this method requires.

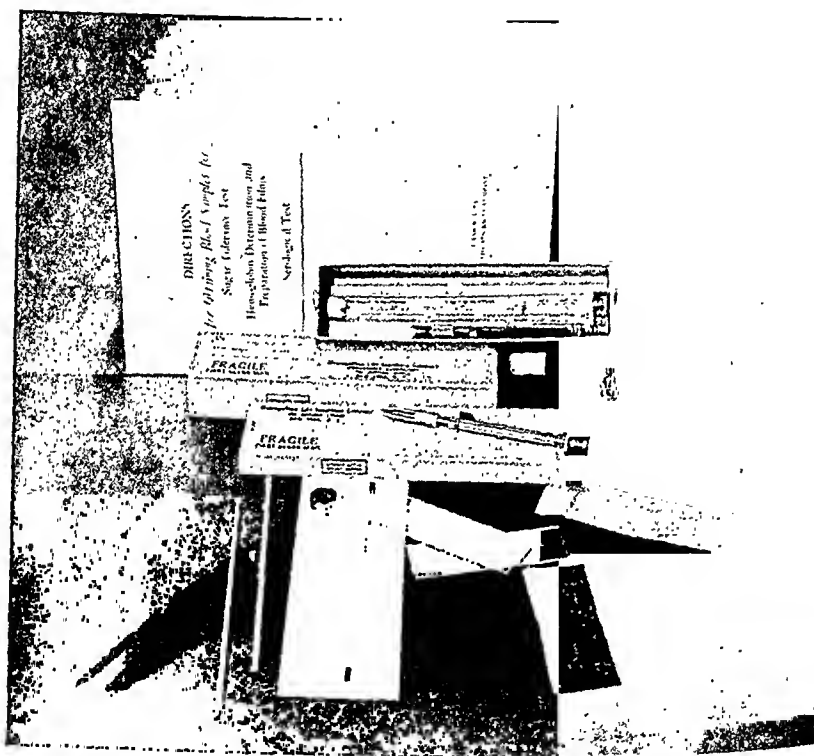


Fig. 1.—Kit for obtaining and mailing small blood samples for hemoglobin determination and examination of blood films. One cellulose acetate tube containing the blood tubes and another holding the lancet are resting in the bottom of the mailer. The inner and outer covers of the mailer are shown. The cap containing the plasticine and the specimen tube sealed with plasticine are resting on the strip of paper. The blood tubes are shown at the left of the latter. The glass slides, glassine envelopes, a lancet, and a copy of the directions are also pictured.

Between the time of collecting and analyzing the blood, a measurable amount of methemoglobin forms at the expense of the oxyhemoglobin. The analysis is therefore, made according to Wu's cyanhemoglobin method¹ which determines both forms of hemoglobin. Oxyhemoglobin is first converted to methemoglobin by adding potassium ferri cyanide, and the methemoglobin is converted to cyanhemoglobin by the addition of potassium cyanide. The same procedure is applied to a standard blood solution which has been adjusted to an oxygen capacity of 4 volumes per cent by the manometric method of Sendroy.² A colorimetric comparison of the blood samples with the standard gives their oxygen capacity.

PREPARATION OF THE BLOOD TUBES

One-fourth gram of potassium oxalate ($K_2C_2O_4 + H_2O$) is dissolved in 2 c.c. of water in a 50 c.c. volumetric flask and 95 per cent ethanol is added until the oxalate is precipitated.

Eight-tenths gram of saponin is mixed with a little alcohol in a small beaker until smooth and rinsed with alcohol into the 50 c.c. flask containing the oxalate. The volume is made up to the mark with alcohol.

After thorough mixing, 2 or 3 c.c. of the alcoholic suspension are poured into a small beaker. The collecting tubes are filled to the mark to which blood is to be collected, and the particles are allowed to settle along the bore. Then a fold of an absorbent towel is applied to the tip of each tube in turn and the alcohol is drawn off as nearly as possible, leaving the oxalate and saponin in the tip of the tube. A slow current of air is then blown through the tip of the tube from a rubber tube, spreading the particles in a thin film in the bore of the tube.

DETERMINATION OF HEMOGLOBIN

A quantity of blood (15 or 20 c.c.) is standardized by the oxygen capacity method and diluted with 0.4 per cent ammonia solution to an oxygen capacity of 4 volumes per cent. This solution may be kept in an amber bottle in a refrigerator for a month or two without appreciable change.

One cubic centimeter of the standard blood solution is delivered into a 20 c.c. volumetric flask and diluted with 4 c.c. of 0.4 per cent ammonia solution.

Two cubic centimeters of 0.4 per cent ammonia solution are placed in a test tube accurately graduated at 10 c.c. One-tenth cubic centimeter of blood is delivered into the tube, and the pipette is rinsed several times with the ammonia solution.

One-fourth cubic centimeter of 0.4 per cent potassium ferricyanide solution is added to the blood sample and 0.5 c.c. to the standard. After twenty minutes 0.25 c.c. of 0.1 per cent potassium cyanide solution is added to the unknown and 0.5 c.c. to the standard. At the end of five minutes both solutions are diluted to the mark with water and the unknown is read against the standard in the colorimeter:

$$\frac{20 S}{U} = \text{Volumes per cent oxygen capacity.}$$

The cyanhemoglobin color is very stable, thus lending itself admirably to photoelectric and photometric determinations. We use the Leifo photometer made by E. Leitz, and for such readings the blood samples are diluted four times more than for colorimetric determination.

EXPERIMENTAL RESULTS

A series of 35 blood samples was kept at room temperature for periods varying from four to nine days, and the results were compared with the original values obtained on freshly drawn samples which contained neither oxalate nor saponin. The results are given in Table I. It will be seen that the figures for the preserved samples are in good agreement with those for the original samples. The values for the kept samples did not vary more than plus or minus 5 per cent

from the original values. During a period of five months we have received 108 blood samples collected by medical examiners in all parts of the country. These were entirely satisfactory for hemoglobin determination. Six samples were not collected satisfactorily. This is a good result when it is recalled that the procedure is a new one for the examiners.

TABLE I
OXYGEN CAPACITY OF FRESH AND PRESERVED BLOOD SAMPLES

SAMPLE	DAYS KEPT	OXYGEN CAPACITY IN VOLUMES PER CENT	
		FRESH SAMPLE	PRESERVED SAMPLE
1	6	20.0*	20.7*
2	6	18.8*	19.2*
3	6	18.4*	17.6*
4	6	21.3*	20.9
5	6	19.0*	18.2
6	6	21.2*	21.6*
7	6	18.4*	18.0*
8	7	19.7	20.6*
9	7	18.5	19.0*
10	7	21.3	20.9
11	7	18.5	17.9
12	7	19.4	19.0
13	7	18.0	17.9
14	7	21.7	22.2
15	7	20.0	19.5
16	7	21.4	20.8
17	7	18.7	19.1
18	7	16.8*	17.5
19	7	17.5*	17.9
20	7	17.5	17.9
21	6	17.9	17.9
22	6	18.5	18.7
23	6	20.5*	20.1
24	6	19.6*	19.7
25	6	19.4*	20.0
26	4	19.7*	20.4
27	4	19.0*	19.2
28	4	20.1	20.9
29	7	17.3	17.1
30	7	17.0	17.9
31	9	18.9	19.0
32	9	16.5	17.4
33	9	16.6	16.3
34	8	19.2	19.5
35	8	20.9	21.3

*This value is an average of duplicate determinations.

SUMMARY

A simple and inexpensive procedure is given for obtaining small blood samples which are mailed to the laboratory from all parts of the country for hemoglobin determination. Similar procedures are also used for blood samples obtained during sugar tolerance tests and for the Kline serologic test.

REFERENCES

1. Wu, H.: Studies on Hemoglobin. I. The Advantage of Alkaline Solutions for Colorimetric Methods for the Determination of Hemoglobin, *J. Biochem.* 2: 173, 1922; Peters, J. P., and Van Slyke, D. D.: *Quantitative Clinical Chemistry*, Methods, Baltimore, 1932, Williams & Wilkins Co.
2. Sendroy, J., Jr.: Manometric Determination of Hemoglobin by the Oxygen Capacity Method, *J. Biol. Chem.* 91: 307, 1931.

A DISTILLING APPARATUS AND A PROCEDURE FOR THE DETERMINATION OF ALCOHOL IN BLOOD AND URINE*

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IOWA CITY, IOWA

THE apparatus permits the rapid distillation of the alcohol from blood or urine and its oxidation to acetic acid with dichromate and sulfuric acid under reflux. Thus the loss of alcohol and of the intermediate oxidation product, acetaldehyde, is avoided. Ground glass connections throughout and ease in cleaning reduce the chances of contamination with organic material to a minimum. The determination is simple. The specimen, mixed in picric acid solution, is distilled (Nieloux)¹ into dichromate solution, sulfuric acid is introduced through the reflux tube for the oxidation, and the excess dichromate is titrated in the receiving flask when cool with a ferrous sulfate-methyl orange mixture (Harger).² Duplicate analyses check usually within 1 per cent with a maximum error of not over 2 per cent.

The apparatus, of pyrex glass, is shown in Fig. 1. Duplicate distillation flasks and three or more receiving flasks are desirable. The total cost should not exceed \$50. A microburner is employed for heating. The apparatus is assembled with clamps and rings on an iron laboratory support stand. The set-up requires but little table space and may be left assembled for use.

ANALYTICAL PROCEDURE

Solutions.—1. Standard dichromate, exactly 2.1288 Gm. of potassium dichromate (analytical) are dissolved in and made up to 1,000 c.c. with distilled water.

2. A saturated solution of picric acid (recrystallized), approximately 12.5 Gm. to 1,000 c.c.

3. Titrating solution, a mixture of 60 c.c. of 50 per cent (by volume) sulfuric acid, 40 c.c. of 0.1 per cent methyl orange in N/40 sodium hydroxide, and 2 c.c. of ferrous sulfate solution (50 Gm. crystals and 30 c.c. of concentrated sulfuric made to 250 c.c.), the mixture to be prepared the day the analysis is done, and cooled to room temperature before using.

The Analysis.—The apparatus and other glassware used are to be chemically clean. Pipette exactly 10 c.c. of the dichromate solution into each of two receiving flasks and into two 250 c.c. Erlenmeyer flasks (to standardize the titration mixture). Introduce 20 c.c. of picric acid solution into each distilling flask along with a few chips of unglazed porcelain. Connect one receiving flask to the condenser. Pipette 1 c.c. of blood (after mixing the specimen) or of urine

*From the Pathological Chemistry Laboratory of the University Hospital, the State University of Iowa.

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distillation and oxidation for a duplicate determination. Next add 10 c.c. of water to each of the blank standard dichromate flasks and 20 c.c. of sulfuric acid, and cool to room temperature. The blanks and the unknowns in the receiving flasks are titrated with the red reducing titration mixture from a burette. The color change is from an orange or yellow-green through a clear green to a solution with a slight reddish tinge. The end point is sharp and most satisfactory.

Calculation.—Ten times the distillate titration is divided by the blank titration, and the result is subtracted from 10.00. The figure thus obtained, when multiplied by 50, gives the milligrams per 100 c.c. of the sample.

The apparatus and procedure described permit certain refinements for the determination of blood and urine alcohol content which should appeal to and will be recognized by the competent analyst. Regardless of the analytical procedure employed, results which are presented as evidence in court should be checked by analysis in duplicate, a customary requirement for reliable commercial testing and for research data. The analytical method outlined above will facilitate such checking.

Acknowledgment is made to Mr. Herman Wiegand of the Department of Chemistry for suggestions as to design and for the construction of this apparatus.

REFERENCES

1. Nicloux, M., LeBreton, E., and Donteheff, A.: Méthode de dosage de quantités d'alcool de l'ordre du dixième de milligramme, *Compt. rend. Soc. de biol.* 116: 79, 1934.
2. Harger, R. N.: Simple Micromethod for Determination of Alcohol in Biologic Material, *J. LAB. & CLIN. MED.* 20: 746, 1935.

A RAPID SIMPLE TEST FOR SULFANILAMIDE AND ITS DERIVATIVES*

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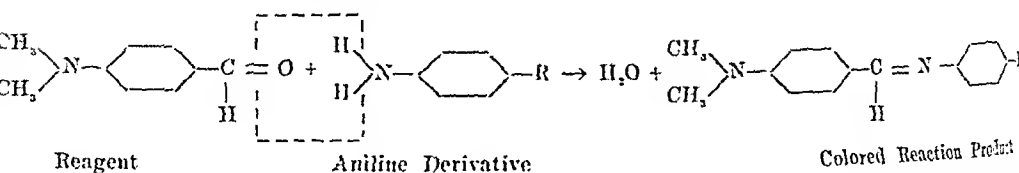
KNOWING the desirability of having an instantaneous simple test which employs only one common, cheap, and stable reagent for drugs of the sulfanilamide group, I wish to report my original application of an old and hitherto overlooked principle of organic chemistry. The reagent is one which most physicians probably already have in their offices. The test is applicable quantitatively to urine and qualitatively to other body fluids.

The tests heretofore, which are all either the original Marshall test or a modification of it, require the diazotization of the amino group on the benzene ring with nitrous acid and the coupling of the resulting compound in acid solution with dimethyl- α -naphthylamine (or a similar compound). An azo dye, usually red or purple, is thus formed and compared with a similarly treated standard solution in a colorimeter. This test, while very accurate and sensitive, requires four reagents, two or three intermediate filtrations, and for even a fast technician at least thirty minutes to perform all the steps and ob-

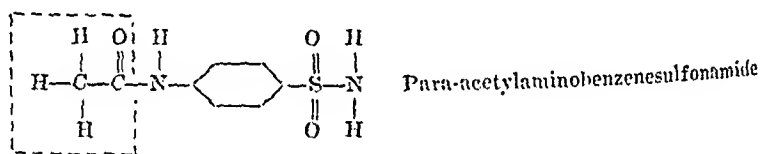
*From the Department of Medicine, Stanford University Medical School, San Francisco. Received for publication, August 2, 1940.

tain complete color development. All this, plus the necessity of having to make up the nitrite solution fresh each time, the expense and often nonavailability of the coupling reagent, and the inevitable high cost and delay in obtaining the desired information, result in such tests being applied much less frequently in ordinary clinical practice than they should be.

In my test the simple addition of para-dimethylaminobenzaldehyde (Ehrlich's reagent) in an acid solution to the tissue fluid produces an immediate bright yellow color. In this test the aldehyde group of the reagent reacts with the free amino group on the benzene ring of the sulfanilamide, sulfapyridine, or sulfathiazole, etc.



In the accompanying structural formula R may be any of numerous radicals in the para position. It may even be a hydrogen atom, for ordinary aniline gives a positive test. In order that the reaction may take place, it is necessary that the NH_2 (amino) group be unchanged; if even one of the H atoms is replaced by another radical, the reaction is blocked. Therefore it is a test for free (unchanged) sulfanilamide. In man these compounds are changed to a varying degree to the monoacetylated form. For example:



If the total (free plus acetylated) amount of these compounds is desired, it is necessary only to heat with hydrochloric acid; in the resulting hydrolysis the acetyl group is split off and the original compound is obtained. Similarly, acetanilid ($\text{CH}_3\text{CO.NH.C}_6\text{H}_5$) does not give the test, but when it is hydrolyzed by heat and acid with the production of aniline ($\text{C}_6\text{H}_5\text{NH}_2$), the test is obtained.

In order to understand the application of the test and its limitations, a brief study must be devoted to possible interfering substances and their elimination. (Most of these interfere with the Marshall test also.)

1. The reagent itself is yellow, and correction must, therefore, be made for it in a quantitative test.

2. Bilirubin (bile pigment) in serum or urine imparts a yellow color to it. Dilution of the urine to 1:200 usually eliminates this interference. If the amount of bilirubin is excessive, it may be precipitated out as insoluble calcium bilirubinate by the addition of calcium chloride solution.

3. Urobilinogen in urine yields a pink color upon addition of the reagent. Dilution of the urine to 1:200 does away with this interference in practically all cases. Shaking the urine with a few drops of hydrogen peroxide solution

to oxidize the urobilinogen to urobilin (which is nonreactive) is an additional precaution; this prevents the pink color formation even in concentrated urine.

4. Other urine pigments can be disposed of by diluting the urine so that there is no perceptible yellowness and then applying the test. The urine may be diluted freely, for the compounds are detectable in a dilution of 1:5,000,000.

5. Some drugs have a free amino group attached to a benzene ring. These would rarely be present without the knowledge of the physician, the drugs in question being procaine and the antisyphilitic arsenicals, arsphenamine, neoarsphenamine, mapharsen, etc.

6. Urea yields a faint yellow color when tested with the reagent, but since the test is about 500 times more sensitive to sulfanilamide than to urea, dilution of the urine or blood filtrate disposes of this interference.

7. Drugs with an acetyl-amino group attached to a benzene ring which yield a free amino-benzene group on acid hydrolysis would interfere in the determination of total sulfanilamide. The only two compounds in this class are acetanilid and acetphenetidin ($\text{C}_{11}\text{H}_{13}\text{NO}_2$). Neither of these compounds is ordinarily administered to a patient being treated with sulfanilamide or its derivatives, the bone marrow already being subjected to sufficient hazard.

8. Sodium nitrite yields a yellow color also, but the amounts administered would rarely if ever be quantitatively significant, and the color reaction is four times more sensitive with sulfanilamide and its related compounds.

9. Alkalinity causes interference because the reaction is obtained only in acid solution. The reagent and sulfanilamide yield no color in an alkaline solution, but the yellow color appears immediately upon acidification. The reagent is, therefore, prepared in concentrated hydrochloric acid as follows:

Para-dimethylaminobenzaldehyde	1.57 Gm.
Concentrated hydrochloric acid, C. P.	20.00 c.c.
Distilled water to make	100.00 c.c.

This formula is very similar to Ehrlich's reagent, which is already universally used as a test for urobilinogen in urine. The aldehyde is much more soluble in acid solution than in neutral solution. The exact proportions of reagent and acid were arrived at by experimentation to obtain the greatest color change in proportion to the color of the reagent itself. After the addition of the reagent the liquid to be tested should be definitely acid to litmus paper. If it is not, hydrochloric acid should be added until it is. This is particularly important with blood plasma and serum which are highly buffered at a pH of about 7.4. Protein precipitation may cause some interference and necessitate filtration. Excess acidification of any fluid is to be avoided because it reduces the color response after a certain point.

10. All other usual drugs, alkaloids, inorganic salts, glucosides, etc., do not interfere. The test was applied to all the remedies mentioned in *Useful Drugs*.

QUANTITATIVE DETERMINATION OF FREE SULFANILAMIDE, SULFAPYRIDINE, AND SULFATHIAZOLE IN URINE

This method employs the Klett-Summerson Photoelectric Colorimeter with its No. 42 (blue) color filter. The results obtained agree exactly with those

obtained by the diazotization methods (after the corrections which the latter require). All figures refer to readings on this instrument with distilled water at zero, and for those who do not possess such a colorimeter the figures obtained can be used as a basis to establish a set of color standards or as a basis upon which the usual colorimeter can be used. Directions are as follows:

1. Fill a Klett tube with distilled water and adjust colorimeter to give zero reading. Discard water.

2. Prepare a 1:200 dilution of the urine. To 10 c.c. of this solution in the Klett tube add one drop of hydrogen peroxide solution and invert several times.

3. Note reading R_n . This usually runs from one to five but may go up to ten.

4. Add exactly 0.2 c.c. of reagent and invert several times.

5. Note reading R_n .

6. Calculate by use of formulas to find concentration in the diluted urine.

Multiply by 200 to get concentration in original urine (undiluted).

$$\frac{R_n - R_n - 98}{7.5} = \text{Parts per million of sulfanilamide}$$

$$\frac{R_n - R_n - 98}{5.18} = \text{Parts per million of sulfapyridine}$$

$$\frac{R_n - R_n - 98}{5.06} = \text{Parts per million of sulfathiazole}$$

The figure 98 is obtained as follows: 0.2 c.c. of reagent in 10 c.c. of distilled water gives a reading of 96 with several brands of pure para-dimethylaminobenzaldehyde that the author has been able to obtain. (It might be well to check your own supply of this compound and adjust the concentration so that this figure is obtained, or to note the value that you obtain yourself.) In a 1:200 dilution of urine the urea present adds 2 to this figure, giving 98. By way of orientation it may be noted that 1:10,000 potassium dichromate (icterus index 1.0) gives a reading of 106.0. The test is most accurate with values in the diluted urine of 2 to 30 parts per million. If more is obtained, the use of a 1:400 dilution is advisable.

If a Klett colorimeter is not available, there are two other alternatives which give satisfactory results. The first is to match the color obtained with a set of permanent (stoppered) standards prepared from potassium dichromate. For example:

0.0094%	$K_2Cr_2O_7$	= 100.0 Klett	= Approx. zero p.p.m.
0.015%	$K_2Cr_2O_7$	= 159.0 Klett	= 7.9 p.p.m. Sulfanilamide
0.02%	$K_2Cr_2O_7$	= 212.0 Klett	= 15.0 p.p.m. Sulfanilamide
0.025%	$K_2Cr_2O_7$	= 265.0 Klett	= 22.0 p.p.m. Sulfanilamide
0.03%	$K_2Cr_2O_7$	= 318.0 Klett	= 29.0 p.p.m. Sulfanilamide

That is, R_n is obtained by comparison, and R_n is arbitrarily set at 2 in using the calculation formulas for the appropriate compound. The other alternative is to compare the color obtained in an ordinary colorimeter with 1:10,000 potassium dichromate, assigning to the latter the value of 106 and obtaining a value for R_n . A value of 2 can be assigned to R_n and the formulas applied.

QUANTITATIVE DETERMINATION OF TOTAL (FREE AND PARA-ACETYLATED) SULFANILAMIDE, SULFAPYRIDINE, AND SULFATHIAZOLE IN URINE

Heat 1 c.c. of urine and 1 c.c. of 2 normal hydrochloric acid in a covered beaker in a water bath (boiling) for thirty to sixty minutes. Let cool. Neutralize to litmus with 2 normal sodium hydroxide. Add water to make up to 200 c.c. Run determination as for free compound omitting the hydrogen peroxide, which is no longer necessary.

APPLICATION OF TEST TO OTHER BIOLOGIC FLUIDS (BLOOD, CEREBROSPINAL FLUID, GASTRIC CONTENTS, EXUDATES, ETC.)

The test is applicable qualitatively, but there are so many uncontrollable factors affecting the final yellowness that quantitative estimations are very unreliable. The more accurate diazotization method is to be preferred for quantitative work on such fluids. In using trichloroacetic acid filtrates of blood it is found that the color response is cut down below a usable level. In trying to use ethereal and alcoholic extracts of blood, we find that the varying color and turbidity plus the yellowness of the reagent cause insuperable difficulties.

NOTE: The solution of the reagent in acid decomposes only very slowly under ordinary conditions. This change can be further minimized by storing the solution in a cold, dark place when it is not in use.

A SIMPLIFIED UREA DETERMINATION FOR ROUTINE BLOOD CHEMISTRY*

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THE determination of urea in blood can be simplified by placing urease, together with oxalate, in specimen bottles, so that the blood, still warm after withdrawal, is in contact immediately with urease. The urea, converted almost instantaneously, can then be determined by direct nesslerization in Folin-Wu filtrates which at the same time serve for the common routine determinations.

TECHNIQUE

One volume of sodium oxalate and two volumes of jack bean powder are mixed by shaking in a large brown bottle which serves as stock container.† For use, the powder mixture is filled into a salt cellar with a 5 mm. opening in the cap‡ and the desired powder quantity obtained by tapping the cap of the salt cellar against the neck of the specimen bottle. (Accurate measuring is not necessary as the blood to be placed into the bottle is, as a rule, not measured either. One milligram of oxalate per 1 c.c. of blood is sufficient; 20 mg. per 10 c.c. of

*From the Long Island College of Medicine Division, Kings County Hospital, Brooklyn. The present work was continued at the Jewish Hospital, Louisville.

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†This powder mixture, using jack bean meal of the Arlington Chemical Co., Yonkers, N. Y., has been kept at room temperature for six months at a time.

‡Suitable are glass salt cellars with screw caps of translucent plastic in which a hole can easily be bored.

blood offers a suitable excess. With a little experience, the volume of mixed powder to be taken is easily recognized.) Immediately after placing blood into the bottle, the latter must be rotated for thirty seconds to insure solution of the oxalate and even distribution of the urease. After five minutes at room temperature the specimen is ready for precipitation: Take 3 c.c. of blood and 24 c.c. of N/12 sulfuric acid, rotate, and set aside for a few minutes. Then add 3 c.c. of 10 per cent sodium tungstate, mix thoroughly, and filter through a 12.5 Whatman No. 1 (yield: about 20 c.c.). If the precipitation is red brown instead of black brown, usually caused by too much oxalate, add a drop of concentrated sulfuric acid. The reagents are best kept in 50 c.c. burettes connected with storage bottles.

For direct nesslerization 3 c.c. of Nessler's reagent and 2 drops of 10 per cent gum acacia are placed in a test tube. Five cubic centimeters of filtrate are blown into the reagent and compared with 5 c.c. of a standard containing 0.00707 per cent of ammonium sulfate (equal to 1.5 mg. per cent N and 0.5 per cent Na_2SO_4). Holding the tip of the pipette at the edge of the test tube, blowing as forcefully as possible, and directing the jet of filtrate directly into the Nessler reagent (prepared according to Boek-Benedict) gives the clearest solutions. Gum acacia (10 per cent in 0.25 per cent benzoic acid) is added to keep the solution stable for at least thirty minutes. The addition of sodium sulfate to the standard gives the same electrolyte concentration as in the filtrate, with an identical color tinge and an identical faint haziness after standing. If necessary the 8 c.c. of unknown solution are diluted with distilled water.

TABLE I

COMPARATIVE ANALYSIS OF BLOOD SAMPLES WITH AND WITHOUT ADDITION OF JACK BEAN POWDER AFTER STANDING 3 TO 5 HOURS AT ROOM TEMPERATURE

ANALYZED FOR	METHOD	NO. OF SAMPLES ANALYZED	MEAN OF RESULTS IN MG. % JACK BEAN POWDER		MEAN OF DIFFERENCES %
			NOT ADDED	ADDED	
Glucose	Folin-Wu	125	118	125	7.1
Creatinine	Folin	52	1.91	1.90	5.5
Uric acid	Benedict	26	3.33	3.11	7.7
Chlorides	Whitehorn	21	469	469	3.2
Nonprotein nitrogen	Folin-Myers	20	36.9	41.7	30.0

Interference.—Before using the described technique generally for routine chemistry, it was necessary to investigate whether or not the addition of jack bean powder to oxalated blood interferes with the common routine tests. It will be seen from Table I that with the exception of nonprotein nitrogen determinations, the mean results obtained in samples without addition of jack bean agree within the limit of methodical error with those obtained in samples with addition of jack bean. Nonprotein nitrogen values in samples containing jack bean powder are too high and cannot be used. However, the addition of jack bean powder to oxalated blood does not interfere with the determination of glucose, creatinine, uric acid, and chlorides in the Folin-Wu filtrate.

Glycolysis.—To prevent glycolysis it is possible to use, instead of oxalate, sodium fluoride, which, on the other hand, retards the urease action. In the presence of sodium fluoride and high urea concentrations the enzymatic reaction

takes about one hour at room temperature and about half an hour in the incubator (see Table II), while in normal blood the reaction is completed in about thirty and fifteen minutes, respectively. High values obtained after twenty-four hours are due to the fact that ammonia is split off from protein as observed also by Sander.

For longer preservation of blood samples, for sugar tolerance tests, in cases of diabetes, etc., the use of sodium fluoride is necessary. If urea is to be determined in specimens containing sodium fluoride, completion of the hydrolysis of urea must be insured by allowing sufficient time for the reaction. For general routine work, however, oxalate is preferable, provided that the error due to glycolysis is minimized by precipitating the blood either after five minutes or after a certain constant time, and allowing for the decrease of glucose concentration.

TABLE II

GLYCOLYSIS AND UREASE ACTION IN OXALATED AND SODIUM FLUORIDE SAMPLES OF THE SAME BLOOD

TIME	OXALATED BLOOD AND JACK BEAN AT 20°		NAP BLOOD AND JACK BEAN AT 20° AT 38°			
	GLUCOSE	UREA	GLUCOSE	UREA	GLUCOSE	UREA
5 min.	81	205	79	26	-	-
15 min.	76	215	-	81	81	86
30 min.	74	198	77	117	80	196
60 min.	74	195	83	197	77	215
120 min.	67	205	78	195	74	205
180 min.	68	215	77	207	79	199
24 hours	50	221	86	212	91	222
48 hours	15	228	90	235	-	-

COMMENT

The method of adding urease to oxalated blood, incubation, and subsequent direct nesslerization of the Folin-Wu filtrate was devised by Myers in 1921. This principle was adopted also by Van Slyke and Plazin, but without incubation, and substituting Somogyi's zinc* for Folin-Wu's tungstic filtration.

The technique of placing oxalate-urease powder directly in specimen bottles, as described, was used in our department during the past year and proved advantageous for the following reasons: (1) Time and labor saving. (2) Eliminates incubation, urease extracts, and buffer. (3) Rapid action of urease. (4) Clear solutions. (5) No interference with routine determinations of glucose, creatinine, uric acid, and chloride.

SUMMARY

A routine urea determination in blood has been described with the technical feature of placing an oxalate-jack bean powder mixture in specimen bottles effecting rapid hydrolysis of urea in the still body-warm blood. The urea is determined by direct nesslerization in the Folin-Wu filtrate which can be used also for other routine tests.

I am indebted to Dr. Hamilton J. Crawford, Director of the Department of Medicine at the Long Island College Division, Kings County Hospital, Brooklyn, and Dr. Carl H. Greene, Clinical Professor of Medicine, for their help and interest in the present work. I wish to thank also Miss Sylvia Held, M.A., for doing part of the analyses.

*Somogyi's copper filtrate likewise gives true sugar values and contains less substances interfering with Nessler's reaction. It is free of uric acid.

REFERENCES

1. De, P., and Bhattacharyya, S.: Glycolysis in Drawn Blood in 24 Hours, *Indian J. M. Research* 25: 933, 1938.
2. Gruskin, B.: A New and Shorter Method for the Determination of Urea and Sugar in Blood, *J. LAB. & CLIN. MED.* 10: 233, 1924.
3. Hawk, F. L., and Andes, J. E.: Simplified Method for Determination of Urea, *Am. J. Clin. Path. Tech. Supp.* 2: 153, 1938.
4. Karr, W. G.: A Method for the Determination of Urea Nitrogen, *J. LAB. & CLIN. MED.* 9: 329, 1924.
5. Looney, J. M.: The Determination of Blood Urea Nitrogen by Direct Nesslerization, *J. Biol. Chem.* 88: 184, 1932.
6. Myers, V. C.: *Practical Chemical Analysis of Blood*, St. Louis, 1921, The C. V. Mosby Co., p. 35.
7. Sander, F. V.: The Preservation of Blood for Chemical Analysis, *J. Biol. Chem.* 58: 1, 1923.
8. Seljeskog, S. R., and Cavett, J. W.: Blood Urea, Comparison of Methods, *J. LAB. & CLIN. MED.* 23: 194, 1937.
9. Shapiro, C. S.: A Direct Nesslerization Method for Urea Nitrogen in Blood Where Commercial Urease Is Employed, *J. LAB. & CLIN. MED.* 19: 649, 1934.
10. Van Slyke, D. D., and Plazin, J.: *Peters and Van Slyke, Quantitative Clinical Chemistry* 2: 558, 1932.
11. Somogyi, M.: The Use of Copper and Iron Salts for the Deproteinization of Blood, *J. Biol. Chem.* 90: 725, 1931.
12. Taylor, W. F., and Blair, W. M.: An Improved Method for the Determination of Blood Urea Nitrogen by Direct Nesslerization, *J. LAB. & CLIN. MED.* 17: 1256, 1932.
13. Wrenn, H. T.: Rapid and Accurate Methods for the Determination of Urea Nitrogen in Blood and Urine by Direct Nesslerization, *J. LAB. & CLIN. MED.* 22: 1040, 1936.

MODIFIED TECHNIQUE FOR PROTHROMBIN DETERMINATION IN BLOOD*

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THE tests for prothrombin content of blood in general use are those of Quick, Brinkhous, and Smith. The determination of prothrombin by the technique of Brinkhous and Smith involves many reagents and is difficult to perform. Quick, however, has evolved a method which is simple, accurate, and inexpensive, but there are several objections to his technique; namely, the reagents must be made up fresh for each determination, the concentration of thromboplastin is variable, and the normal time value for the method is too short. This favors error in reading and does not permit the measurement of prothrombin levels above normal.

It was felt that these objections to Quick's method were valid and that a modified test might be evolved which would overcome them. Early in our experimental work we investigated several sources of thromboplastin, namely, dried brain tissue from the rat, guinea pig, rabbit, sheep, and man. The variation in results obtained with these sources of thromboplastin is shown in Table I. All tests were performed according to the technique of Quick.¹²

Subsequently the effect of varying the concentration of thromboplastin, plasma, and calcium chloride in different combinations was determined. These findings, as shown in Table II, may be summarized as follows: (1)

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Increasing the proportion of plasma decreases the prothrombin time. (2) Increasing the proportion of thromboplastin decreases the prothrombin time except in the case of thromboplastin from the rat. (3) There is an optimum concentration of calcium chloride for each type of thromboplastin. Increasing or decreasing this concentration prolongs the prothrombin time. (4) There is an optimal concentration of all reagents for each type of thromboplastin.

TABLE I

AVERAGE VALUES ARE GIVEN WITH STANDARD DEVIATIONS FOR EIGHT BLOOD SAMPLES

THROMBOPLASTIN FROM	PROTHROMBIN TIME (SECONDS)
Rat brain	87 ± 2.8
Guinea pig brain	201 ± 10.3
Rabbit brain	32 ± 0.9
Sheep brain	118 ± 6.2
Human brain	72 ± 1.8

The tests were made with optimum concentration of reagents (see Table II).

After a series of preliminary experiments and primarily because of its stability, an ether-soluble, acetone-insoluble extract of sheep brain was chosen as a thromboplastic agent for use in a modified technique. Its preparation is described subsequently.

MODIFIED TECHNIQUE

1. Plasma from oxalated blood is used. A mixture of 4.5 c.c. of blood and 0.5 c.c. of sodium oxalate gives sufficient plasma for several determinations. To separate the plasma from the cells, the blood is centrifuged for fifteen to twenty minutes at high speed to throw down as much of the tissue debris and as many cellular elements as possible.

2. Into a small glass tube (serology tubes 3 by $\frac{9}{16}$ inches and 4 by $\frac{1}{2}$ inches are adequate) 0.2 c.c. of plasma is accurately pipetted with a Kahn pipette. To this is added 0.2 c.c. of the thromboplastin solution. (Stock solution is diluted with normal saline 1:5 on day of test.)

3. The mixture is incubated at 37° C. in the water bath for seven minutes.

4. The tube is then removed from the water bath, the outside of the tube is dried, and exactly 0.2 c.c. of calcium chloride is *immediately* added.

5. The tube is then shaken to insure adequate mixing. It is slanted and rotated at fifteen-second intervals. The interval between the addition of the calcium chloride and clot formation is measured with a stop watch. This constitutes the prothrombin time.

REAGENTS

1. *Sodium Oxalate*: 1.34 Gm. of C. P. anhydrous sodium oxalate are dissolved in 100 c.c. of distilled water. This solution is tenth-molar and is nearly isotonic.

2. *Thromboplastin Stock Solution* (ether-soluble, acetone-insoluble brain extract): A fresh sheep brain is washed in water. The meninges and blood vessels are carefully removed. The brain tissue is then cut into pieces as small as possible and is dried with acetone. Repeated additions of acetone and grinding in a mortar facilitate this process. The final product is freed of acetone by centrifuging, carefully decanting the acetone, and drying the powder in the

TABLE II
TESTS WERE RUN BY QUICK'S METHOD. ALL RESULTS INDICATE AVERAGES AND STANDARD DEVIATIONS OF AT LEAST EIGHT DETERMINATIONS

NO.	RATIO			PROTHROMBIN TIME (SECONDS) AND SOURCE OF THROMBOPLASTIN				
	PLASMA	THROMBO-PLASTIN	CALCIUM CHLORIDE	RAT	GUINEA PIG	RABBIT	SHEEP	MAN
1	1	1	M/10	242 ± 5.4	525 ± 9	65 ± 2.5	576 ± 19.1	153 ± 7.4
2	2	1	M/10	137 ± 3.4*	296 ± 27	40 ± 1.4	259 ± 12.7	109 ± 4.8
3	0.5	1	M/10	593 ± 17	1094 ± 16.3	169 ± 7.7	1110 ± 140	309 ± 8.5
4	1	2	M/10	349 ± 17†	398 ± 2.3	46 ± 1.2	436 ± 16	158 ± 8.8
5	1	0.5	M/10	251 ± 6.4	1124 ± 144	71 ± 1.8	740 ± 56	168 ± 6.4
6	1	1	M/10	560 ± 24.6‡	2080 ± 149	161 ± 3.8	1380 ± 97	422 ± 11.7
7	1	1	M/20	157 ± 1.9	291 ± 19	32 ± 0.9	248 ± 11.7	75 ± 3.66
8	1	1	M/40	109 ± 1.6	201 ± 10.3§	41 ± 1.8	138 ± 3.8	72 ± 1.8
9	1	1	M/80	94 ± 2.6	243 ± 18.7	34 ± 0.2	142 ± 3.8	73 ± 3.4
10	1	1	M/160	88 ± 3.3	290 ± 7.8	32 ± 0.9	118 ± 6.2§	120 ± 15.3
11	1	1	M/320	87 ± 2.8§	320 ± 11.8	35 ± 1.1	185 ± 27.2	158 ± 19.2
12	1	2	M/20	224 ± 7.2	297 ± 15	48 ± 2.1	154 ± 7.2	94 ± 8.1
13	2	1	M/80	90 ± 3.2	220 ± 10.6	42 ± 3.2	233 ± 28.4	94 ± 4.4

*Note effect of doubling quantity of plasma. (Compare lines 1 and 2.)

†Note effect of doubling thromboplastin. (Compare lines 1 and 4.)

‡Note effect of doubling calcium chloride. (Compare lines 1 and 6.)

§Note optimum concentration of calcium chloride. This optimum is only apparent with rat, guinea pig, and sheep thromboplastin. With rat, rabbit, and human thromboplastin note the range in which the concentration of calcium chloride has but slight effect (indicated by brackets).

open air. The dry powder is then completely extracted with repeated small portions of ether until no precipitate is obtained by addition of acetone. To the combined portions of the ethereal extract, acetone is added until no more of the extracted material can be precipitated from the solution. The precipitated substance is separated from the ether-acetone mixture by centrifugation. It is then air-dried and kept in a stoppered vial in the icebox. The finished product is a light brown, waxy substance which darkens on standing. A normal yield from an average sheep brain is about 5 to 10 Gm. In the preparation of the stock solution 0.25 Gm. of the substance is emulsified in 50 c.c. of normal saline, and aqueous merthiolate is added as a preservative to a concentration of 1:100,000. The saline suspension is warmed to 56° C. for ten minutes to facilitate mixing, and during this time it is thoroughly shaken. The finished product is a homogeneous opalescent mixture which for test purposes is diluted 1:5 with normal saline. We have kept the stock preparation in the refrigerator for as long as three to five months without the slightest evidence of deterioration. Brief experiments with a similar extract from rabbit brain revealed that it gave practically the same values for prothrombin time as did thromboplastin from the sheep brain.

3. *Calcium Chloride*: 1.11 Gm. of C. P. anhydrous calcium chloride is dissolved in 800 c.c. of distilled water, giving a final concentration of eightieth-molar.

All reagents except the thromboplastin are stored at room temperature.

DISCUSSION

The essential factors involved in such a test are multiple, each in turn having its separate influence upon the clotting phenomenon. Any test concerning the clotting mechanism must attempt to keep the variability of these factors at a minimum. The following techniques have been found most important and difficult to standardize:

The proportion of oxalate to whole blood is very important, and the ratio of 1:10 should be strictly adhered to. Experimentally, doubling the quantity of oxalate will increase the prothrombin time 200 per cent. Varying differences in prothrombin times may be obtained by concentrations between these extremes. Slight hemolysis in the sample of blood does not alter the prothrombin time appreciably, but gross hemolysis does shorten it sufficiently to invalidate the determination.

Although it is desirable to determine the prothrombin time of a sample as soon as possible, oxalated whole blood will usually keep satisfactorily in the icebox for twenty-four hours without material alteration in the level of prothrombin if the plasma is not separated from the cells. Individual samples of blood have been kept as long as seventy-two hours, with an alteration in prothrombin content of only about 15 per cent. Recent work in connection with blood stored in "blood banks" indicates that most blood shows a marked deterioration of prothrombin within twenty-four hours and that it is almost depleted in forty-eight to seventy-two hours. The rate of destruction or fixation is probably an individual characteristic and cannot be predetermined.

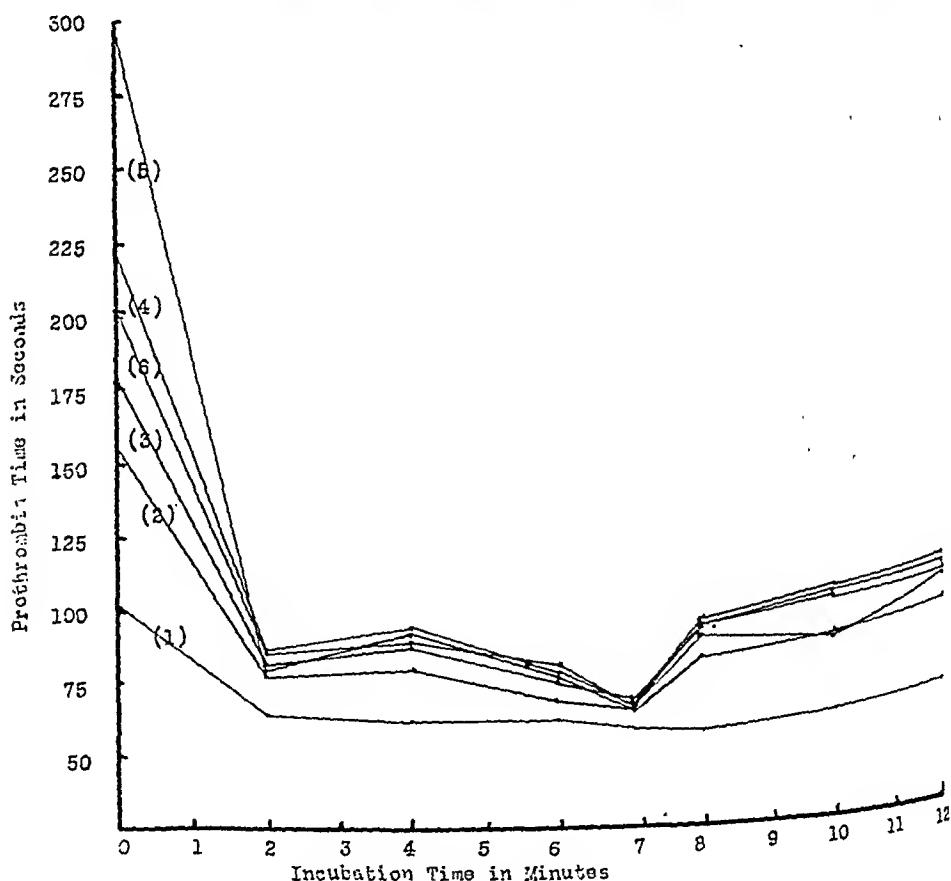
It is important that the concentration of all reagents, and particularly neutral salts, be maintained at a uniform level. Normal saline must be used

in the thromboplastic solution because an excess of sodium chloride will materially delay the clot formation. This phenomenon is not universal, and individual samples of blood vary in their susceptibility to this change.

TABLE III
TEST AS OUTLINED, USED IN ALL CASES

INCUBATION PERIOD (MINUTES)	PROTHROMBIN TIME (SECONDS)						SPREAD	AVERAGE
	CASE							
	1	2	3	4	5	6		
0	101	153	176	222	290	202	121	173.6
2	66	78	82	86	80	85	20	79.5
4	65	82	88	95	93	89	30	83.3
6	65	71	77	80	79	83	18	74.3
7*	61	67	74	72	69	70	13*	65.8*
8	58	79	87	92	84	86	34	82.6
10	65	88	99	102	87	96	37	89.5
12	68	93	104	108	94	98	40	94.2

*Note minimum spread and shortest prothrombin time.

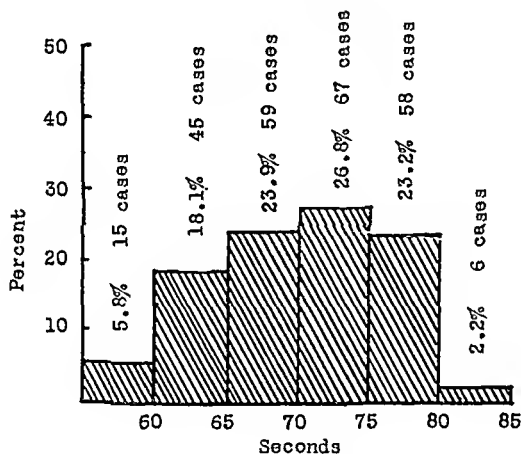


In the reading of the test the end point is taken as the time when the mixture first begins to show a fibrin web after gentle periodic rotation. Many times this point is coincidental with complete clot formation. Again it may precede complete clotting by ten to twenty seconds. Violent agitation of the tube after addition of the calcium chloride will delay the clot formation.

During the formation of a clot it has been determined that the pH of the solution is only slightly altered toward the alkaline side. Samples of serum before, during, and after clot formation do not vary appreciably in their pH range. Consequently in most instances it is not necessary to regulate this factor since the normal buffer systems existing in the blood plasma are sufficient to keep the pH within normal limits under conditions of the test.

TABLE IV

TIME GROUP (SECONDS)	CASES	PER CENT
55-60	15	5.8
60-65	45	18.1
65-70	59	23.9
70-75	67	26.8
75-80	58	23.2
80-85	6	2.2
Total	250	100.0



The incubation period is the time interval during which the mixture of thromboplastin and plasma stands before the addition of the calcium chloride. Table III shows the alteration in prothrombin times with varying periods of incubation. The period of incubation which gave the shortest prothrombin time and the most constant results was found to be seven minutes. The decrease in prothrombin time to an optimum at seven minutes' incubation is probably due to the maximum release and formation of thrombin from the plasma. It is probably necessary for a given concentration of thrombin to be formed before it can act as an enzyme in the clotting mechanism. Although it is well known that, given sufficient time, a very small amount of thrombin can convert large quantities of fibrinogen to fibrin, there is apparently an optimum concentration at which it acts. The spread of individual determinations is probably due to the separate rates of release of thrombin or its precursor, prothrombin. Why there is an increase in prothrombin time with longer than seven minutes'

incubation is not easily understood, since thrombin and prothrombin are believed to be very stable. Some light may be thrown on this question by the work of Mertz, Seegers, and Smith,²⁶ who have suggested that an antiprothrombin is formed which may be thrombin itself.

In all tests involving colloidal chemistry unexplained variations commonly occur. If this fact is not recognized, considerable error may creep into their interpretation. For this reason, it is advisable to evaluate a normal control with each sample of supposed pathologic blood. It is also advisable, unless there is other clinical evidence of a prothrombin alteration, that all abnormal values be checked with repeated specimens of blood before therapy is instituted.

CLINICAL EVALUATION

For purposes of clinical evaluation of this test samples of blood from 250 consecutive patients admitted to the Cottage Hospital were tested. None of these patients had clinical evidence of alteration in their blood prothrombin level. The spread of the entire 250 determinations was from a minimum of 55 seconds to a maximum of 85 seconds. Table IV indicates that the range of normal values for this test lies between 60 and 80 seconds, with an average of 70 seconds since 92 per cent of the cases fall in this bracket. The determinations of the prothrombin time can be converted to per cent of prothrombin by considering the normal or 100 per cent to have a prothrombin time of 70 seconds.

We also used this test as a diagnostic procedure on two patients who were suspected of having lowered prothrombin levels of their blood. An omentopexy was performed on one patient with advanced cirrhosis. During his convalescence this patient developed bleeding from his gums and gastrointestinal tract. Prothrombin determinations were made, and an initial value of 187 seconds was found. Therapy with vitamin K reduced this to 95 to 100 seconds, with a marked cessation in bleeding within a period of twenty-four hours. A second patient had marked jaundice of an obstructive nature and an elevated prothrombin time of nearly 200 seconds. Subsequent treatment with vitamin K reduced this to 85 seconds in the course of three days.

SUMMARY

Due to the objections to the present tests in use for the determination of prothrombin in blood, a modified technique has been evolved which overcomes these defects and in addition has the essential factors of adaptability, simplicity, accuracy, and economy. A new thromboplastic agent which is stable and easily prepared has been made from sheep brain. The modified technique has been adequately tested clinically and has been found to be satisfactory.

REFERENCES

1. Quick, A. J., Stanley-Brown, M., and Bancroft, F. W.: Study of Coagulation Defect in Hemophilia and in Jaundice, *Am. J. M. Sc.* 190: 501, 1935.
2. Bancroft, F. W., Kugelmass, I. N., and Stanley-Brown, M.: Evaluation of Blood Clotting Factors in Surgical Diseases, With Special Reference to Thrombosis and Embolism and Certain Bleeding Conditions, *Ann. Surg.* 90: 161, 1929.
3. Linton, R. R.: Relation of Blood Fibrin to Hemorrhagic Diathesis of Obstructive Jaundice, *Ann. Surg.* 96: 394, 1932.
4. Scott, F. H., and Chamberlain, C.: Calcium and Coagulation of Blood, *Proc. Soc. Exper. Biol. & Med.* 31: 1054, 1934.

5. Ferguson, J. H.: Observations on Alterations of Blood Platelets as Factor in Coagulation of Blood, *Am. J. Physiol.* 108: 670, 1934.
6. Ravdin, I. S., Riegel, C., and Morrison, J. L.: Coagulation of Blood, *Ann. Surg.* 91: 801, 810, 1930.
7. Wangensteen, O. H.: Hemorrhagic Diathesis of Obstructive Jaundice and Its Treatment, *Ann. Surg.* 88: 845, 1928.
8. Nygaard, K. K.: Coagulability of Blood Plasma; Remarks on Technique of Its Determination, *Proc. Staff Meet., Mayo Clin.* 9: 151, 1934.
9. Howell, W. H., and Cekada, E. B.: Cause of Delayed Clotting of Hemophilic Blood, *Am. J. Physiol.* 78: 500, 1926.
10. Bancroft, F. W., Quick, A. J., and Stanley-Brown, M.: Anticoagulants of Blood, *Proc. Soc. Exper. Biol. & Med.* 32: 164, 1934.
11. Warner, E. D., Brinkhous, K. M., and Smith, H. P.: Plasma Prothrombin Levels in Various Vertebrates, *Am. J. Physiol.* 125: 296, 1939.
12. Quick, A. J.: Nature of Bleeding in Jaundice, *J. A. M. A.* 110: 1658, 1938.
13. Smith, H. P., Warner, E. D., and Brinkhous, K. M.: Prothrombin Deficiency and Bleeding Tendency in Liver Injury (Chloroform Intoxication), *J. Exper. Med.* 66: 801, 1937.
14. Warner, E. D., Brinkhous, K. M., and Smith, H. P.: Quantitative Study on Blood Clotting; Prothrombin Fluctuations Under Experimental Conditions, *Am. J. Physiol.* 114: 667, 1936.
15. Quick, A. J.: On Action of Heparin and Its Relation to Thromboplastin, *Am. J. Physiol.* 115: 317, 1936.
16. Ferguson, J. H.: Quantitative Relationship of Calcium and Cephalin in Experimental Thrombin Formation, *Am. J. Physiol.* 123: 341, 1938.
17. Ferguson, J. H.: Blood Calcium and Calcium Factor in Blood Coagulation, *Physiol. Rev.* 16: 640, 1936.
18. Quick, A. J.: Coagulation Defects in Sweet Clover Disease and in Hemorrhagic Chick Disease of Dietary Origin; Consideration of Source of Prothrombin, *Am. J. Physiol.* 118: 260, 1937.
19. Quick, A. J.: On Various Properties of Thromboplastin (Aqueous Tissue Extracts), *Am. J. Physiol.* 114: 282, 1936.
20. Smith, H. P., Warner, E. D., and Brinkhous, K. M.: Lung Extract in Blood Clotting, *Am. J. Physiol.* 107: 63, 1934.
21. Snell, A. M.: Symposium on Hemorrhagic Diathesis in Cases of Jaundice: Its Relation to Vitamin K Deficiency; Preliminary Report: Clinical and Experimental Conditions Associated With Deficiency of Prothrombin, *Proc. Staff Meet., Mayo Clin.* 13: 65, 1938.
22. Nygaard, K. K.: Coagulability of Blood Plasma, *Proc. Staff Meet., Mayo Clin.* 7: 544, 1932.
23. Ziffren, S. E., Owen, C. A., Hoffman, G. R., and Smith, H. P.: Control of Vitamin K Therapy. Compensatory Mechanisms at Low Prothrombin Levels, *Proc. Soc. Exper. Biol. & Med.* 40: 595, 1939.
24. Owen, C. A., Hoffman, G. R., Ziffren, S. E., and Smith, H. P.: Blood Coagulation During Infancy, *Proc. Soc. Exper. Biol. & Med.* 41: 181, 1939.
25. Quick, A. J., and Grossman, A. M.: Prothrombin Concentration in Newborn, *Proc. Soc. Exper. Biol. & Med.* 41: 227, 1939.
26. Mertz, E. T., Seegers, W. H., and Smith, H. P.: Inactivation of Prothrombin by Purified Thrombin Solutions, *Proc. Soc. Exper. Biol. & Med.* 41: 657, 1939.

AN INEXPENSIVE ALTERNATING CURRENT PHOTOELECTRIC COLORIMETER*

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A RECENT communication¹ described an inexpensive direct-reading photoelectric colorimeter suitable for clinical use and designed to operate from small, portable dry batteries. In some circumstances the use of an instrument operated from alternating current may be more convenient. Alternating current colorimeters that regulate their light source potential by means of so-called constant-voltage transformers, however, have not been entirely satisfactory. Their erratic performance can be traced to line-voltage variations, not completely minimized by the regulating devices that were available. Recently transformers with adequate control have been developed, making possible the modification of our battery colorimeter for use with alternating current without loss of stability or precision. The new model (Fig. 1) can be constructed easily by anyone familiar with electrical apparatus from parts obtainable at a very low cost from any radio or electric supply house.

DESIGN

The colorimeter shown schematically in Fig. 2 operates as a direct-reading instrument. A solution whose concentration is to be determined is subjected to the usual chemical procedure prerequisite to a colorimetric examination. A sample of the resultant preparation is placed in a test tube, and light from a small incandescent lamp is projected through it. The transmitted light, after passing through a selective color filter, falls on the sensitive surface of a photoelectric cell; in response to the radiation a small electric current is generated. The current is amplified many times by a vacuum tube amplifier and is recorded by a milliammeter. This reading is transposed into terms of solution concentration by a graph prepared after calibrating the instrument with samples of known concentration.

The light source, *L. S.*, is a lens-end type, No. 222 Mazda flashlight bulb. This lamp by its unique construction provides abundant light at relatively low filament temperatures, thereby assuring long filament life and freedom from undesirable heating effects.

The selective color filters are of the Wratten gelatin variety and are obtainable from the Eastman Kodak Co. We have found four types to suffice for all colorimetric examinations usually encountered in the clinical laboratory. These are the blue, No. 47, used in blood sugar determinations; the green, No. 55,

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used in hemoglobin, nonprotein nitrogen, urea nitrogen, uric acid, and creatinine determinations; the yellow, No. 8, used in calcium and phosphorus determinations; and the red, No. 25, used in cholesterol determinations. The choice of the proper filter for other determinations will be discussed later.

The photoelectric cell, Ph , is a rubidium-coated R. C. A. type 926 phototube. Its spectral characteristics make it especially suitable for colorimetry. Although its current response to incident light is extremely small, expensive galvanometers or microammeters are not needed in its circuits because of the tube's adaptability to current amplifier circuits.

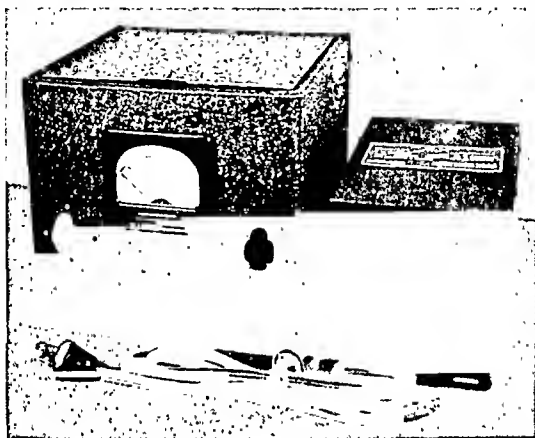


Fig. 1.—The colorimeter. The control on the left changes selective color filters; that on the right operates the power switch and regulates light source intensity.

The amplifier is a single stage, twin triode type. The two triodes of an R. C. A. type 6F8-G radiotron, V_1 , are arranged in a balanced Wheatstone bridge circuit in which plate resistances occupy two of the arms; R_4 , a 100-ohm potentiometer, and R_b and R_L , 1,000-ohm, 0.5-watt resistors, occupy the remaining two. The balance of the bridge is controlled by R_4 . The phototube current flows through R_c , a 20-megohm, 0.5-watt resistor; the potential developed across it is applied directly to the grid of one of the triodes. To maintain the amplifier in a state of balance at all times, R_g , another 20-megohm, 0.5-watt resistor, is connected between the grid of the other triode and ground. Proper grid potentials are obtained by R_a , a 200-ohm, 0.5-watt resistor. A 0.1 milliammeter, $M. A.$, connected across the amplifier bridge, measures the amplified phototube current. A 0.02-microfarad condenser, C_s , inserted across R_c , slows the action of the milliammeter.

The great stability of a twin triode amplifier makes it preferable to other types. Several were set up in the laboratory and operated continuously for 500 hours. All remained balanced during this time, even though line-voltage fluctuations often were as great as 10 per cent. When the power to an amplifier

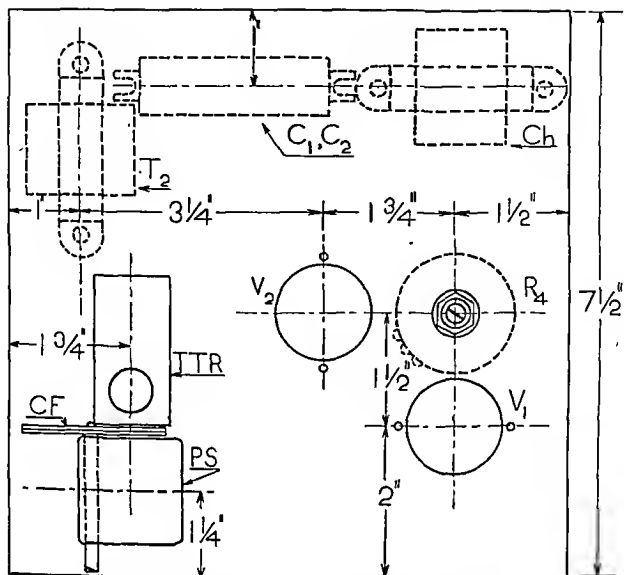


Fig. 3.—The subpanel layout. C_1 and C_2 , filter condenser block; CF , selective color filter assembly; Ch , filter choke; PS , phototube shield; R_4 , amplifier balancing potentiometer; T_2 , 6.3 volt transformer; TTR , test tube receptacle; V_1 , amplifier tube socket, V_2 , rectifier tube socket.

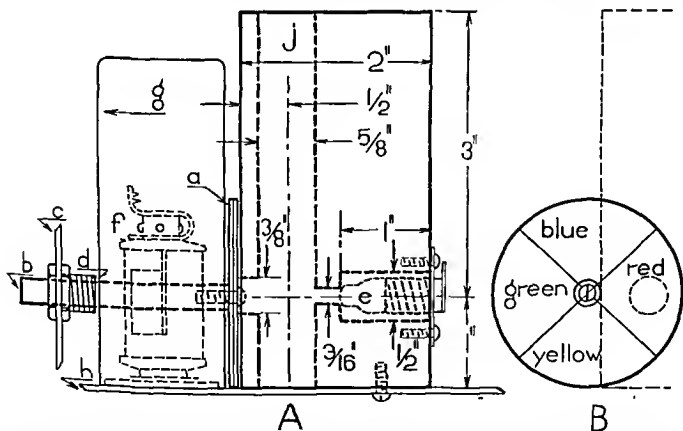


Fig. 4.—A, optical system; a, circular glass plates with interposed selective color filters; b, filter control shaft; c, front panel; d, shaft bearing; e, projection chamber containing flash-light lamp; f, phototube and socket; g, phototube shield; h, subpanel; j, test tube receptacle B, arrangement of selective color filters between circular glass plates.

ometer, R_2 , are mounted on the front panel; the remaining parts are placed inside the cabinet on a subpanel and arranged as in Fig. 3.

The structural details of the optical system are shown in Fig. 4A. An aluminum block, 4 by 2 by 1 inches, drilled according to the specifications outlined, forms a projection chamber for the flashlight lamp and a receptacle in which test tubes containing solutions to be examined can be inserted. The flashlight lamp is mounted in a miniature screw base socket firmly bolted to the block. Light passes from the projection chamber to the test tube receptacle through an opening $\frac{3}{16}$ inch in diameter; another opening, $\frac{3}{8}$ inch in diameter, on the opposite side of the receptacle, conducts transmitted light to the phototube, mounted directly in front of it within a metal shield, $3\frac{1}{2}$ by $1\frac{3}{8}$ by $1\frac{3}{8}$ inches, to exclude stray light.

The four selective color filters are mounted between two circular glass plates, 2 inches in diameter and $\frac{1}{16}$ inch in thickness, having holes $\frac{5}{32}$ inch in diameter drilled through the centers and sealed at the edges with Duco cement (Fig. 4B). This filter assembly is placed between the aluminum block and phototube shield and is bolted to a brass shaft, 3 inches long and $\frac{1}{4}$ inch in diameter, supported by a bearing attached to the front panel of the colorimeter. By rotating the shaft the operator can quickly move any of the four filters into the path of the light beam.

OPERATION

Balancing the Bridge.—When the colorimeter has been assembled, the first procedure is to balance the amplifier bridge. The flashlight lamp is removed from its socket, the line cord plugged into an alternating current outlet, and the potentiometer, R_2 , rotated to the right. In the first few degrees of rotation, the switch, Sw , is closed and the power turned on. Before any adjustments are made, the colorimeter should operate continuously for twenty-four hours. Since new amplifier tubes require several hours of heating to become stabilized, efforts to balance the bridge permanently before stability is attained are wasted. When the prescribed time has elapsed, the potentiometer, R_2 , mounted on the subpanel of the instrument, is rotated until the milliammeter needle is brought accurately to its zero scale position. The amplifier circuit is then balanced and needs to be readjusted only if an amplifier tube is changed. When the flashlight lamp is replaced in its socket, the colorimeter is ready for operation.

Matching the Absorption Tubes.—Ordinary soft glass test tubes, 5 or 6 inches long and $\frac{5}{8}$ inch in outside diameter, are used as absorption cells. To obtain a set of tubes of identical light transmission, it is suggested that several dozen be purchased, cleaned carefully with sulfuric acid-dichromate cleaning mixture, and filled with distilled water. A sample tube is placed in the instrument, the power turned on, and the potentiometer, R_2 , rotated until the milliammeter needle is brought to any convenient position near full scale. The meter deflection produced by each of the tubes is noted, and those whose readings deviate more than 0.5 of 1 per cent from the mean are discarded. The remaining ones are kept for colorimetric use.

Choosing a Selective Color Filter.—Photoelectric colorimeters measure the percentage light absorption of solutions submitted for examination. The re-

lationship between light absorption and solution concentration can be expressed, according to the Beer-Lambert law, by the equation,

$$\log \text{ per cent absorption} = KCX,$$

where C is the concentration of the solution examined, X is the length of the solution through which light passes, and K is a constant. For a particular set of matched absorption tubes, X is a constant; therefore, the concentration is proportional to the logarithm of the light absorbed or inversely proportional to the logarithm of the light transmitted. The value of K depends upon the wave length of the light transmitted by the solution. In some portions of the spectrum the absorption is relatively small; in regions corresponding to the positions of the solution's characteristic absorption bands it is much greater. When various selective color filters that transmit light only in limited regions of the spectrum are used, the absorption can be made to have a wide range of values. It obviously is desirable not to have the absorption too small, for then it becomes difficult to measure. On the other hand, it is evident by differentiating the equation that, when the absorption becomes too great, large changes in concentration produce very small changes in light absorption, equally difficult to estimate. Satisfactory performance is attained if the absorption is restricted to less than 70 per cent of the incident light. Generally, a filter of the color complementary to that of the solution examined fulfills these conditions. In some instances, however, as for example in the Folin-Wu determination of blood sugar, the color density of the solution is so great that a filter having a color similar to that of the solution should be used. Regulation can be achieved in other ways, namely, by altering the amount of substance taken for analysis or by changing the volume of the final dilution.

When the proper filter is to be selected, a solution is prepared having the maximum concentration desired to be determined. With a test tube of distilled water in the receptacle, the meter is set at full scale deflection by the potentiometer, R_2 . Then the distilled water is replaced by the colored solution, and the readings are noted with each of the four filters. That solution which produces a deflection not exceeding about 70 per cent of the full scale is chosen for the particular determination.

Making a Determination.—A series of standard solutions having concentrations ranging from the lowest to the highest values expected to be encountered in the laboratory is prepared. A solution having zero concentration is also included. With the proper filter in place and a tube of distilled water in the receptacle, the meter is set at full scale deflection. The distilled water is then replaced in turn by each of the standard solutions and the meter reading of each recorded.

Calibration of the instrument can be carried out in several different ways. One method is to determine the specific extinction coefficient, $E_{sp.}$, in the equation,

$$E_{sp.} = \frac{\log \frac{T_0}{T}}{C},$$

where T_0 is the meter reading of the solution having zero concentration, T is the meter reading of one of the standard solutions, and C is its concentration. The mean of the various values of the coefficient can then be used to calculate the concentration of any solution according to the formula,

$$C = \frac{\log \frac{T_0}{T}}{E_{sp.}}$$

A more direct method of calibrating the colorimeter is simply to make a graph plotting solution concentrations against the logarithms of the meter readings. A line is drawn through the points, and concentrations can be read directly from the graph. An improvement of this method is to plot the graph on semilogarithmic paper, with meter readings placed along the logarithmic axis. Observations then can be converted directly to concentration values without calculation. Once the calibration curve is made, it is unnecessary to prepare standard solutions in making subsequent determinations, except as an occasional check or when new reagents are prepared. When a determination is made, the power to the instrument is simply turned on; two minutes are allowed for the amplifier and flashlight lamp to heat; the proper selective color filter is inserted; the potentiometer, R_2 , is adjusted for a full scale deflection of the meter with a test tube of distilled water in its receptacle; and the reading of the specimen is observed. The concentration is evaluated from the calibration curve.

If a series of consecutive determinations is to be made, it is advisable from time to time to check the position of R_2 . Fatigue of the light source causes a slow drift of the meter needle, making slight readjustments necessary. When the colorimeter has been in operation for ten minutes, the rate of drift is 0.25 of 1 per cent per minute.

In addition to the general principles of operation outlined, no attempt will be made to discuss the application of the instrument to specific problems. Many recently published reports describe the use of direct-reading photoelectric colorimeters in a great many clinical laboratory procedures.

SUMMARY

An inexpensive photoelectric colorimeter suitable for clinical use and designed to operate from alternating current outlets is described. Complete details of design, construction, and operation are included. Performance comparing favorably with that of commercial colorimeters can be expected. Operation is extremely simple.

REFERENCE

1. Morgan, R. H., and Weinhouse, S.: An Inexpensive Portable Photoelectric Colorimeter, *Am. J. Clin. Path.* 10: 114, 1940.

THE PHOTELOMETRIC DETERMINATION OF BILIRUBIN IN URINE WITH DIAZOBENZENESULFONIC ACID*

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A SIMPLE, rapid and accurate procedure for the quantitative estimation of urinary bilirubin has been developed for use with the Sheard-Sanford photometer. This instrument makes use of spectral filters selected to transmit light chiefly in the region of maximal absorption of the substance under test, and thereby deletes the effects of extraneous colored substances or other regions of spectral absorption which may be present in the solution. The photometer also eliminates personal error in color matching and dispenses with the need for artificial standards.

The photometer is calibrated for bilirubin in the manner described by Osterberg, except that it is not necessary to evaporate the solution of bilirubin in chloroform to dryness. A weighed sample of bilirubin is dissolved in chloroform, and serial dilutions corresponding to convenient concentrations of bilirubin are prepared. Two milliliter portions of these solutions, to each of which is added a small quantity of alcohol, are treated with 1 ml. of the diazo reagent and are made up with alcohol to a final volume of 10 ml. These solutions are compared with water in the photometer.

For the calibration in terms of bilirubin, a spectral filter that has its maximal transmission in the region of 530 to 550 $m\mu$ is chosen. It is most satisfactory to construct the calibration curve on semilogarithmic paper; this makes it easy to select any desired scale value (such as 50) as the reading which corresponds to a solution free of bilirubin. Less than full scale deflection may be desired when small amounts of solution are to be examined; for, if light passes above the meniscus of the solution, the accuracy of the readings is lost.

METHOD

A method for the determination of urinary bilirubin is herein proposed.

Solutions.—1. *Caffeine sodio-benzoate and buffer solution.* Mix equal parts of a 25 per cent solution of caffeine sodio-benzoate† in distilled water and Sørensen's phosphate buffer pH 6.6. The buffer is most conveniently prepared‡ by mixing 60 ml. of one-fifth molar potassium dihydrogen phosphate (13.617 Gm. of KH_2PO_4 in 500 ml. of water), 40 ml. of one-fifth molar dibasic sodium phosphate (17.814 Gm. of $Na_2HPO_4 \cdot 2H_2O$ in 500 ml. of water), and 200 ml. of water.

*From the Mayo Clinic, Rochester.

†Received for publication, March 25, 1940.

‡Caffeine sodio-benzoate (U. S. P. X) is defined as "a mixture of caffeine and sodium benzoate which contains, when dried to a constant weight at 80° C., not less than 47 per cent nor more than 50 per cent of anhydrous caffeine ($C_8H_{10}O_2N_4$), and not less than 50 per cent nor more than 53 per cent of sodium benzoate ($NaC_6H_5O_2$)"

§Modified from the description of Clark.

2. *Acid blank solution.* Dilute 7.5 ml. of concentrated hydrochloric acid with water to make one liter.

3. *Diazo reagent.* Note that although this diazo reagent contains the same substances as Ehrlich's diazo reagent and many of its modifications (such as the so-called van den Bergh reagent), the concentrations are less in the one described here.

I. Sulfanilic acid ($\text{C}_6\text{H}_4(\text{NH}_2)(\text{SO}_3\text{H}) + 2\text{H}_2\text{O}$) 1 Gm., concentrated hydrochloric acid 15 ml., and water to make one liter.

II. Sodium nitrite (NaNO_2) 0.5 Gm. and water to make 100 ml. For use, take 0.3 ml. of solution II to 10 ml. of solution I, and dilute with water to make 20 ml.

PROCEDURE

To each of two tubes, "blank" and "test," add 2 ml. of fresh centrifuged urine and 2 ml. of caffeine sodio-benzoate and buffer solution (1). Mix well by rotating the tubes, and let stand for ten minutes or a little longer. To tube "blank" add 1 ml. of acid blank solution (2) and 5 ml. of water. To tube "test" add 1 ml. of diazo reagent (3) and 5 ml. of water.

Let both tubes stand for ten to fifteen minutes. With the same filter which was used in calibrating the photometer in place and the contents of the "blank" tube in the test cell, adjust the instrument to the scale value which corresponds to a solution free of bilirubin, and then substitute the contents of tube "test" in another cell. From the scale reading and the calibration curve, determine the content of bilirubin. If the content of bilirubin in the urine is too high to be read within the range of the instrument, the urine may be diluted with water, and the test repeated.

SPECTROPHOTOMETRIC AND PHOTELOMETRIC EXPERIMENTS

With methods hitherto employed for the quantitative determination of urinary bilirubin, it has been necessary to resort to adsorption of the bilirubin to an inert precipitate (Hunter,⁵ Naumann, Jendrassik, and Grof) in order to avoid interference from other urinary substances. In the case of the procedure which we have described, this same result is achieved through control of the pH of the reaction mixture and use of the urine (a colored solution), properly diluted, as a "blank" for adjusting the photometer before taking a reading. To demonstrate the validity of this procedure, we have made certain spectrophotometric studies of solutions of bilirubin, urine, and bilirubin in urine.

If a sample of urine from a jaundiced patient is treated with caffeine sodio-benzoate, diazobenzenesulfonic acid in hydrochloric acid solution,* and is buffered to a final pH of 4 to 5.5, a definite red tint appears. The spectrophotometric comparison of such a solution with water in terms of percentage of light transmitted at various wave lengths is shown in Fig. 1 (curve a). A similar comparison of the same urine treated with caffeine sodio-benzoate, buffer, and an amount of hydrochloric acid equivalent to that in the diazo reagent (that is, the "blank" solution already described) is shown in Fig. 1 (curve b). The sample to which diazo reagent has been added transmits much less light in the

*Hereinafter designated the "diazo reagent."

region between 500 and 600 $m\mu$ than does the untreated sample. In the region between 450 and 520 $m\mu$, however, various urinary pigments cause a considerable reduction in the light transmitted by the untreated sample. An allowance for this absorption, common to the two samples, is made by comparing the sample to which the diazo reagent has been added with the sample untreated (except for identical acidification, buffering and dilution), instead of with water. The resulting percentage transmission of light at various wave lengths is shown in Fig. 1 (curve *c*); there has appeared, as the result of the addition of the diazo reagent, a distinct zone of increased spectral absorption with a maximal value between 520 and 540 $m\mu$. The spectrophotometric measurement of the light transmitted by a solution of purified bilirubin treated with the diazo reagent in a mixture of chloroform, alcohol, and water is represented in Fig. 1 (curve *d*) and is essentially identical in character and spectral region of maximal absorption with that represented in Fig. 1 (curve *c*).

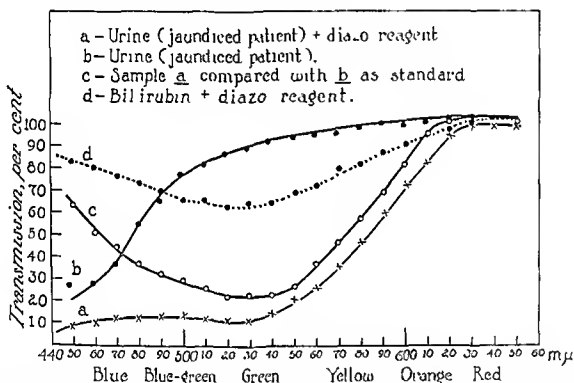


Fig. 1.—Spectrophotometric transmission curves: *a*, of urine (diluted 1:4) from a jaundiced patient after the addition of acetate buffer, diazo reagent, and water, compared with water as a standard; *b*, of urine (diluted 1:4) from the same sample after the addition of acetate buffer, acid blank solution, and water, compared with water as a standard; *c*, of the solution represented by curve *a* when compared with the solution represented by curve *b* instead of water as a standard; *d*, of pure bilirubin dissolved in chloroform, diluted with alcohol, treated with the diazo reagent, and compared with water.

In Fig. 2 (curve *a*) is shown the spectrophotometric curve of light transmitted by a mixture of phosphate buffer, alcohol, diazo reagent, and bilirubin dissolved in sodium carbonate solution (compared with water). A portion of the same sample of bilirubin dissolved in sodium carbonate solution was added to normal urine. A portion of the resulting mixture was treated with caffeine sodio-benzoate, buffer, and the diazo reagent, and was compared spectrophotometrically with another portion of the mixture treated with caffeine sodio-benzoate, buffer, and hydrochloric acid only. Fig. 2 (curve *b*) shows the percentage transmission of light then obtained at various wave lengths. Both the spectrophotometric curves obtained for bilirubin in chloroform and carbonate solutions after treatment with the diazo reagent, and the curve obtained after adding the same re-

agent to bilirubin in urine (using the untreated urine as a standard) are of the same general character and exhibit the same zone of absorption, with maximal absorption at 520 to 540 $m\mu$.

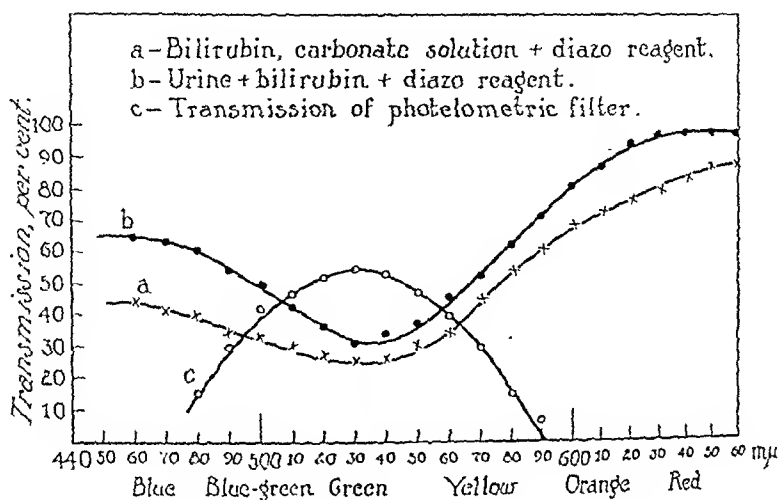


Fig. 2.—Spectrophotometric transmission curves; a, obtained when bilirubin dissolved in a solution of sodium carbonate is treated with the diazo reagent in the presence of phosphate buffer (pH 6.6) and alcohol, and compared with water as a standard; b, obtained when a mixture of normal urine and bilirubin dissolved in a solution of sodium carbonate is treated with caffeine sodio-benzoate, phosphate buffer, and diazo reagent, and compared with the same mixture treated with caffeine sodio-benzoate, phosphate buffer, and acid blank solution as a standard. Curve c represents the percentage of incident light transmitted at various wave lengths by the photometer filter used in these experiments.

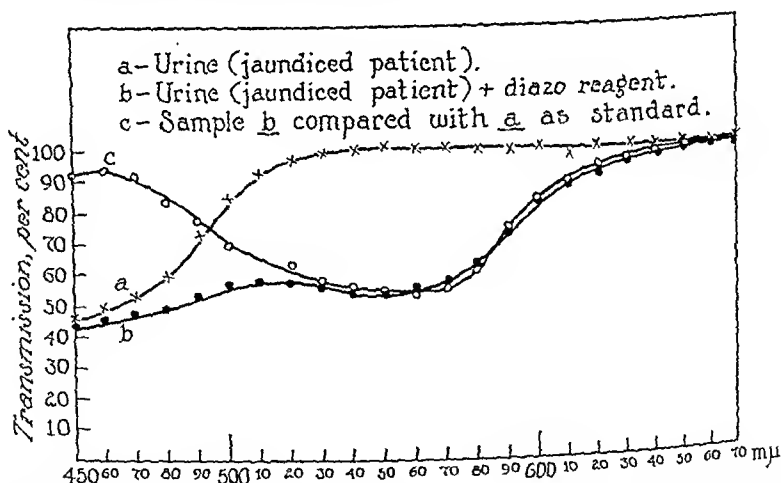


Fig. 3.—Spectrophotometric transmission curves; a, of urine from a jaundiced patient, treated with caffeine sodio-benzoate, phosphate buffer, and acid blank solution, as described in the text, and compared with water as a standard; b, of a sample of the same urine treated with caffeine sodio-benzoate, phosphate buffer, and diazo reagent, as described in the text, and compared with water as a standard; c, of the solution represented by curve b when compared with curve a, instead of water, as a standard.

Oxyhemoglobin has bands of spectral absorption with their maximal values at 542 and 578 $m\mu$, respectively, and, therefore, it might be presumed that blood would interfere with the photometric determination of bilirubin. A specimen of urine from a jaundiced patient was divided into two portions: to the first

*Intact erythrocytes, of course, would be removed by centrifugation, along with other debris, from any sample ordinarily submitted to the test.

was added a quantity of hemolyzed blood,* and to the second an exactly equal amount of distilled water. The curves of Figs. 3 and 4 show how the use of the untreated urine blank compensates for the presence of added hemoglobin. In Fig. 3 curve *a* represents the spectrophotometric transmission of the portion diluted with water, prepared as for the "blank" solution, and compared with water; curve *b* contains the data on the same sample after treatment with the diazo reagent as already described; and curve *c* shows the resultant percentage transmission of light when the treated urine is compared with the untreated urine as a standard. In Fig. 4 curve *a* represents the spectrophotometric transmission

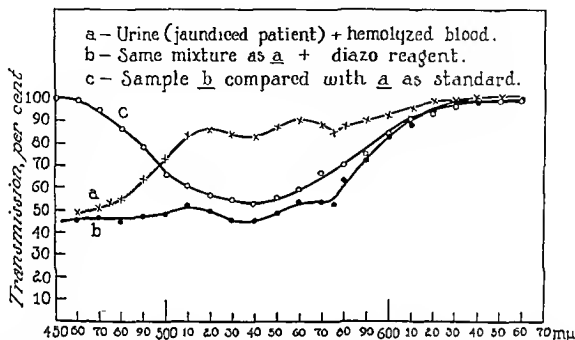


Fig. 4.—Spectrophotometric transmission curves: *a*, of urine from a jaundiced patient (the same sample as used for the curves of Fig. 3), with added hemolyzed blood, treated with caffeine sodio-benzoate, phosphate buffer, and acid blank solution, and compared with water as a standard, *b*, of the same urine-hemolyzed blood mixture treated with caffeine sodio-benzoate, phosphate buffer, and diazo reagent, and compared with water as a standard, *c*, of the solution represented by curve *b* when compared with the solution represented by curve *a* as a standard, instead of with water.

for the portion to which the hemolyzed blood was added and demonstrates the typical absorption curve of oxyhemoglobin superimposed on that of the urine alone; curve *b* represents the spectrophotometric transmission of the urine-hemoglobin mixture after the addition of the diazo reagent (compared with water); and curve *c* represents the spectrophotometric transmission of the urine-hemoglobin-diazo mixture compared with the untreated urine-hemoglobin mixture. It is obvious that curve *c* in Fig. 4 is essentially identical with curve *c* in Fig. 3.

Specimens of urine from several jaundiced patients were divided and treated as just described, and were then assayed for bilirubin in the photometer. The data are presented in Table I; they clearly demonstrate that the added hemolyzed blood does not impair the accuracy of our method.

We have shown by the foregoing experiments (a) that the development of typical diazobilirubin is not hindered by urinary constituents, and (b) that under the conditions which we have specified, diazobenzenesulfonic acid does not couple with urinary constituents other than bilirubin to produce a colored substance having a zone of absorption in the region between 500 and 600 $m\mu$. We have also shown that pre-existing urinary pigments which produce some absorp-

tion in the spectral region with which we are concerned (that is, 520 to 540 $m\mu$) are entirely compensated for by the use of the diluted acidified urine, untreated with the diazo reagent, as a standard for spectrophotometric or photometric comparison.

TABLE I
EFFECT OF ADDING HEMOLYZED BLOOD TO URINE

NUMBER	MILLIGRAMS OF BILIRUBIN PER 100 ML. OF	
	URINE	URINE + HEMOLYZED BLOOD
1	3.20	3.20
2	1.30	1.35
3	0.70	0.70
4	1.03	1.03
5	1.70	1.65

When spectrophotometric determinations are made of the transmissions of normal urine before (Fig. 5, curve *a*) and after (Fig. 5, curve *b*) treatment with the diazo reagent, both identically diluted and compared with water, it is again apparent, as in the case of urine from a jaundiced patient (Fig. 1, curves *a* and *b*) that there is a greater absorption of light in the region of the shorter wave lengths after the addition of the diazo reagent. When the sample to which the diazo reagent has been added is compared spectrophotometrically with the untreated sample, however, no characteristic band of absorption appears (Fig. 5, curve *c*). This is probably due to an overshadowing effect of the unchanged urinary pigments. Since the photometer deletes the effect of the increased absorption which appears to be produced in the deep blue region, this may be disregarded. We know of no other naturally occurring substance in the urine which could react with the diazo reagent under these circumstances to develop the color characteristic of diazobilirubin. Because the transmission curves for the normal urine coupled with the diazo reagent are themselves inconclusive, and because with larger amounts of bilirubin the characteristic absorption band of diazobilirubin develops, we are justified in the conclusion that the maximal absorption of light in the region of 520 to 540 $m\mu$ which occurs in urine treated with diazobenzenesulfonic acid as already described is due to bilirubin.

The importance of maintaining an acid reaction when assaying urine for bilirubin as we have described follows from a consideration of the coupling reactions known to occur with diazobenzenesulfonic acid in sodium carbonate solution. This reaction was introduced by Panly, and together with its modifications has been extensively applied by a number of investigators (Clifford,⁸ Hanke and Koessler,¹⁰ Hunter,^{6, 7} Nakayama,¹³ Hewitt, and others) to many substances, notably the phenols, imidazoles, and certain amino acids. Several of these are included among the seventy possible urinary constituents described by Thierfelder. A few of them, biliverdin, urobilin (Ehrlich), xanthine, hypoxanthine, guanine (Hunter⁶), phenol, and histamine have been specifically tested with the diazobenzenesulfonic acid reagent in acid solution and have been found to produce no color which could interfere with the test for bilirubin. Hunter⁷ stated that aromatic amines (of which there are none in Thierfelder's list) will react

with diazobenzenesulfonic acid in acid solution, but he added that "there has never been any question of its specificity in biological fluids for bilirubin." We feel that this conclusion is strongly confirmed by the spectrophotometric curves which we have presented.

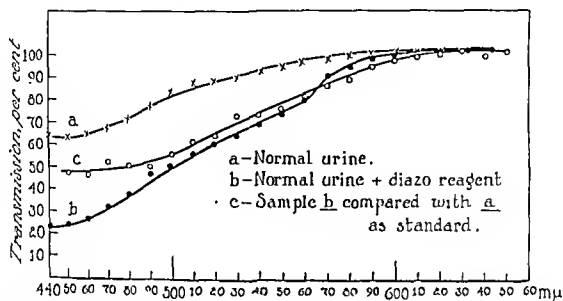


Fig. 5.—Spectrophotometric transmission curves: *a*, of normal urine treated with caffeine acid blank solution, and compared with water as a standard; *b*, of normal urine treated with caffeine sodio-benzoate, phosphate buffer, and the diazo as a standard; *c*, of the solution represented by curve *b* compared with curve *a* as a standard, instead of water.

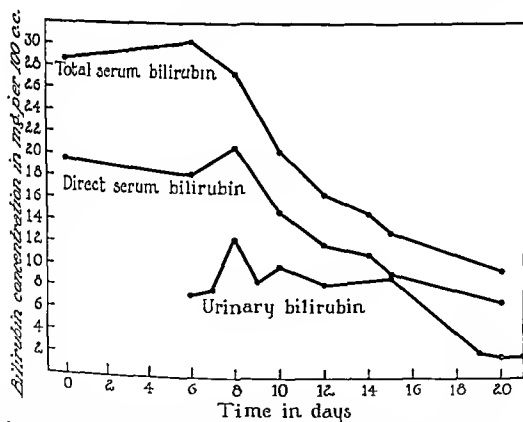


Fig. 6.—Curves representing postoperative fall in total serum bilirubin and urinary bilirubin, expressed in milligrams per 100 ml., over a period of time measured in days. The data were obtained from a case of obstructive jaundice due to carcinoma of the head of the pancreas. The serum bilirubin values were determined by the method of Malloy and Evelyn.

The "Ehrlich diazo reaction," as described in current texts on clinical pathology (for instance, Todd and Sanford), is made in ammoniacal solution. Just what substance produces the test is not definitely known, although it has been studied by Hunter,⁷ Sachs and Schmidinger, Miyazaki, and Nakayama. What we have said about the Pauly reaction applies equally well to the "Ehrlich diazo reaction."

The phosphate buffer which we have included in our reaction mixture serves chiefly to prevent the coagulation of albumin by the acid diazo reagent and to maintain the final pH in the range (4 to 5.5) most favorable for the characteristic red color of diazobilirubin.

Although alcohol is generally relied on to insure complete coupling of the diazo reagent with bilirubin in blood serum, it is unsatisfactory for this purpose in urine, because hazy solutions usually result, even though there be no precipitation of albumin, and in many instances the development of color is inhibited.

TABLE II

ACTION OF ALCOHOL ON THE DEVELOPMENT OF DIAZOBILIRUBIN COLOR IN URINE
(Values expressed in milligrams per 100 ml. of urine)

METHOD	NUMBER	DILUTION	
		WITH ALCOHOL	WITH WATER
I	1	0.27	0.47
	2	0.29	0.43
	3	0.15	0.33
	4	0.35	0.12
	5	0.51	0.53
	6	0.51	0.53
	7	0.13	0.63
	8	0.24	0.37
	9	0.31	0.45
	10	0.08	0.15
II	11	0.03	0.07
	11a	0.15	0.38
	11b*	0.08	0.43
	12	0.31	0.49
	13	0.43	0.33
III	14	0.43	0.78
	15	0.37	0.43
	16	0.40	0.47
	17	0.26	0.38
	18	0.36	0.48

Method I: 5 ml. of methanol or water plus 1 ml. of diazo reagent (or acid blank solution), plus 4 ml. of urine; centrifuge.

Method II: same as Method I, except that the urine was acidified with hydrochloric acid to pH 5.5 to 6 before adding the test solutions.

Method III: 2 ml. of centrifuged urine, plus 2 ml. of phosphate buffer pH 6.6; plus 1 ml. of diazo reagent (or acid blank solution), plus 5 ml. of water or methanol.

*Buffered to pH 6.6 with phosphate buffer before the test.

The generally higher values of the aqueous solutions shown in Table II probably reflect the greater accelerating action of urea, which Adler and Strauss discovered in an effort to determine the nature of the "direct" and "indirect" van den Bergh reactions in blood serum. They found that caffeine sodio-benzoate, urea, and certain other diuretic substances greatly accelerated the development of a maximal degree of color by the diazobenzenesulfonic acid coupling reaction with bilirubin. Caffeine sodio-benzoate in 25 per cent solution can be added to urine without the formation of a precipitate and, as shown in Table III, frequently aids in the development of the maximal depth of color and in the prevention of haziness. Fig. 2 (curve *b*) shows that this color is the same as the color developed in other solutions which contain bilirubin. Because of the inevitable presence of urea in urine, the terms "direct" and "indirect" can have no significance as applied to urinary bilirubin.

TABLE III

EFFECT OF CAFFEINE SODIO-BENZOATE ON THE DEVELOPMENT OF DIAZOBILIRUBIN COLOR IN URINE
(Values expressed in milligrams of bilirubin per 100 ml. of urine.)

NUMBER	WITHOUT CAFFEINE	WITH CAFFEINE
19	0.30	0.55
20	0.30	0.40
21	0.99	0.70
22	0.66	0.70
23	1.57	1.57
24	1.25	1.35
25	0.78	0.93
26	Hazy	3.90

Solutions without caffeine were treated as follows: 2 ml. of urine, 2 ml. of phosphate buffer, 1 ml. of diazo reagent (or acid blank solution) and 5 ml. of water. Solutions with caffeine were treated as described in the text.

TABLE IV

QUANTITATIVE RECOVERY OF BILIRUBIN ADDED TO URINE

SAMPLE	MILLIGRAMS OF BILIRUBIN PER 100 ML. OF				PERCENTAGE RECOVERED*
	URINE	BILIRUBIN SOLUTION	URINE + BILIRUBIN		
			ANTICIPATED	FOUND	
1	0.58	1.08	1.66	1.55	93.5
2	0.55	1.17	1.72	1.63	96.0
3	0.47	1.06	1.53	1.45	94.8
4	0.51	1.09	1.60	1.75	109.4
5	0.37	1.09	1.46	1.50	102.7
6	0.40	0.90	1.30	1.23	96.2
7	0.45	0.90	1.35	1.15†	85.1
8	0.23	0.92		1.15	100.0
9	0.53	9.30	9.83	9.30	94.6
10	0.60	3.10	3.70	3.78	102.1

*Average recovery 97.4 per cent; range 85.1 to 109.4 per cent.

†This solution became faintly hazy on the addition of the bilirubin to the urine.

The final test of any analytical procedure is the accuracy of its quantitative recovery of the test substance. The application of this inquiry to bilirubin in urine is vastly complicated by the virtual insolubility of bilirubin except in highly alkaline mixtures or in organic substances which are immiscible with water. After many experiments it was found that bilirubin dissolved in a solution of urea and sodium carbonate, a mixture* prepared and furnished through the generosity of Dr. David Klein, Director of the Wilson Laboratories, could be added to urine in most instances without precipitation and without making the solution sufficiently alkaline to invalidate the test. The procedure was as follows: 1 ml. of the diluted bilirubin solution was placed in each of two 10 ml. volumetric flasks, and was made up in the one instance with urine and in the other with distilled water to the volume of 10 ml. In a third 10 ml. volumetric flask were placed 1 ml. of distilled water and 9 ml. of urine. From each of these mixtures, 2 ml. were then taken for testing according to the method that we have described, the diluted urine being used as a "blank" for the urine alone and the urine plus bilirubin, and distilled water as the "blank" for the bilirubin

*The original mixture contained in each milliliter 22 mg. of bilirubin, 300 mg. of urea, and 10 mg. of sodium carbonate. This solution is made up at the rate of 300 mg. of urea and 22 mg. of bilirubin per 100 ml. The average daily excretion of urea in normal urine is such that a concentration of at least 1,000 mg. per 100 ml. is maintained. It may be that even though the urine is acid, the bilirubin excreted by the kidneys is held in solution in some way by the urea present.

alone. On the average, as shown by the results in Table IV, 97.4 per cent of the added bilirubin was recovered.

The test here described was developed in connection with certain experiments on the excretion of bilirubin in which a quantitation of urinary bilirubin seemed desirable. In Fig. 6 are shown the relative concentrations of serum bilirubin and urinary bilirubin from a patient whose obstructive jaundice was subsiding postoperatively.

SUMMARY

On the addition of an acid solution of diazobenzenesulfonic acid to urine that contains bilirubin, the typical color of diazobilirubin is developed. The use of a suitable phosphate buffer prevents the precipitation of albumin and maintains the pH of the reaction mixture in the zone between 4 and 5.5. Caffeine sodio-benzoate may be added to assure the development of the maximal depth of color and to aid in the prevention of haziness. It is emphasized that the Pauly reaction and the "Ehrlich diazo reaction" take place in alkaline solution.

Spectrophotometric curves show that the red color developed in urine on the addition of caffeine sodio-benzoate, phosphate buffer, and acid solution of diazobenzenesulfonic acid can be used to estimate bilirubin in the photometer, provided the instrument is standardized against a similarly diluted sample of the untreated urine.

REFERENCES

1. Adler, Erich, and Strauss, Leo: Beitrag zum Mechanismus der Bilirubinreaktion im Blut, *Klin. Wchnschr.* 1: 2285, 1922.
2. Clark, W. M.: The Determination of Hydrogen Ions. An Elementary Treatise on Electrode, Indicator and Supplementary Methods; With an Indexed Bibliography on Applications, ed. 3, Baltimore, 1928, Williams & Wilkins Co., 715 pp.
3. Clifford, Winifred M.: A Method for the Colorimetric Estimation of Carnosine, *Biochem. J.* 15: 400, 1921.
4. Ehrlich, P.: Sulfodiazobenzol, ein Reagens auf Bilirubin, *Centralbl. f. klin. Med.* 4: 721, 1883.
5. Hewitt, L. F.: The Diazo Reaction in Uraemic Sera, *Biochem. J.* 19: 171, 1925.
6. Hunter, George: Estimation of Carnosine in Muscle Extract—A Critical Study, *Biochem. J.* 16: 640, 1922.
7. Hunter, George: The Diazo Reaction in Urine, *Biochem. J.* 19: 25, 1925.
8. Hunter, George: A Diazo Method for Detecting Bilirubin in Urine, *Canad. M. A. J.* 23: 823, 1930.
9. Jendrassik, L., and Grof, P.: Verfahren zur photometrischen Bestimmung des Bilirubins im Harn, *Biochem. Ztschr.* 296: 71, 1938.
10. Koessler, K. K., and Hanke, M. T.: Studies on Proteinogenous Amines. II. A Microchemical Colorimetric Method for Estimating Imidazole Derivatives, *J. Biol. Chem.* 39: 497, 1919; VII. The Quantitative Colorimetric Estimation of Histidine in Protein and Protein-Containing Matter 43: 527, 1920; VIII. A Method for the Quantitative Colorimetric Estimation of Histamine in Protein and Protein-Containing Matter 43: 543, 1920; XIV. A Microchemical Colorimetric Method for Estimating Tyrosine, Tyramine, and Other Phenols, 50: 235, 1922.
11. Malloy, H. H., and Evelyn, K. A.: The Determination of Bilirubin With the Photoelectric Colorimeter, *J. Biol. Chem.* 119: 481, 1937.
12. Miyazaki, Misaki: Studies on the Diazo-Body in Typhoid Urine, *J. Biochem.* 18: 379, 1933 (Abstr.); The Diazo Compound in Typhoid Urine, *Chem. Abstr.* 28: 819, 1934.
13. Nakayama, Sadajiro: Zur Kenntnis des Diazoharns: III. Über die chemische Zusammensetzung des Diazoharns bei Scharlach, *J. Biochem.* 19: 33, 1934 (Abstr.); Diazo Urines. III. Chemical Composition of Diazo Urines in Scarlatina, *Chem. Abstr.* 28: 2405, 1934; IV. Über die chemische Zusammensetzung des Diazoharns bei Masern, *J. Biochem.* 19: 39, 1934 (Abstr.); IV. Chemical Composition of Diazo Urines in Measles, *Chem. Abstr.* 28: 2405, 1934; V. Über die Diazoreaktion des Pockenarns, *J. Biochem.* 21: 439, 1935 (Abstr.); V. Diazo Reaction of the Urine From Smallpox, *Chem. Abstr.* 29: 8123, 1935; VI. Zur Kenntnis der Antoxypro-

- teinsäure, J. Biochem. 21: 443, 1935 (Abstr.); VI. Studies on Antoxyproteic Acid, Chem. Abstr. 29: 8123, 1935.
14. Naumann, H. N.: A New Test for Bilirubin in the Urine and Its Use for the Detection of Bilirubin in Normal Urine, Biochem. J. 30: 762, 1936.
 15. Osterberg, A. E.: The New van den Bergh Reaction for the Determination of Serum Bilirubin Utilizing the Photometer, J. Lab. & Clin. Med. 22: 729, 1937.
 16. Pauly, H.: Über die Konstitution des Histidins, Ztschr. f. physiol. Chem. 42: 508, 1904.
 17. Pharmacopeia of the United States of America, Tenth Decennial Revision, Philadelphia, 1925, J. B. Lippincott Co., 626 pp.
 18. Sachs, Paula, and Schmidinger, Ludwig: Über die chemische Grundlage der Ehrlichschen Diazoreaktion im Urin. IV. Mitteilung. Stufenphotometrische, quantitative Bestimmung des "blauen Farbstoffes," Ztschr. f. klin. Med. 132: 73, 1937.
 19. Sanford, A. H., Sheard, Charles, and Osterberg, A. E.: The Photometer and Its Use in the Clinical Laboratory, Am. J. Clin. Path. 3: 405, 1933.
 20. Thierfelder, Hans: Handbuch der physiologisch- und pathologisch- chemischen Analyse für Ärzte und Studierende, Berlin, 1924, Julius Springer, 1004 pp.
 21. Todd, J. C., and Sanford, A. H.: Clinical Diagnosis by Laboratory Methods. A Working Manual of Clinical Pathology, ed. 9, Philadelphia, 1939, W. B. Saunders Co., 841 pp.

DEPARTMENT OF ABSTRACTS AND REVIEWS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

GRANULOMAS, Venereal and Non-Venereal of the Vulva, von Haam, E. J. A. M. A. 114: 291, 1940.

The macroscopic and microscopical pictures of 155 cases of infectious granulomas of the vulva were observed in the laboratory for venereal diseases in New Orleans.

Because of the fundamental difference in diagnosis and therapeutics, a subdivision of infectious granulomas into the groups of venereal and nonvenereal lesions is recommended.

The presence of several etiologic factors in one pudendal granuloma could be proved in 11 cases. Various tests for the correct diagnosis of granulomas of mixed etiology have been evaluated.

Proper treatment of a granuloma of the vulva should be deferred until a complete diagnosis of the lesion has been reached. This will be greatly facilitated by the liberal use of laboratory methods.

THYROID DISEASE, A New Diagnostic (Galactose) Test for, Althausen, T. L., Lockhart, J. C., and Soley, M. H. Am. J. M. Sc. 119: 342, 1940.

A new clinical test for activity of the thyroid gland based on the rate of intestinal absorption of galactose is described. It consists of oral administration of galactose followed by determinations of galactose in the blood thirty and sixty minutes later.

Data from 121 control subjects and 130 patients with hyperthyroidism show that the average maximal concentration of galactose in the blood of patients with hyperthyroidism was three times greater than normal and that clinically the test was comparable in reliability to estimations of the basal metabolic rate.

Following thyroidectomy, the galactose tolerance test was normal in almost all cases.

Advantages of the galactose test are that it is more sensitive than the basal metabolic rate in cases of low-grade hyperthyroidism, and that its outcome is not influenced by hyperventilation in anxiety states or by cardiac dyspnea. A disadvantage of the test is that the presence of hepatic insufficiency or of Paget's disease interferes with its use for the diagnosis of thyroid disease.

In myxedema abnormally low galactose tolerance curves were observed, indicating that the galactose test can be used also in the diagnosis of this condition.

The technique of the test follows:

Forty grams of galactose (Pfanstiehl) dissolved in 400 c.c. of water and flavored with lemon juice were administered by mouth after the patient had fasted overnight. Specimens of blood were obtained from the cubital vein before, and 5, 15, 30, 60, and 120 minutes* after administration of galactose.

The glucose fraction of the blood was removed by fermentation with ordinary yeast, according to the method of Somogyi as modified by Raymond and Blanco.

Preparation of Yeast.—A weighed amount of fresh commercial yeast (Fleischmann's) is suspended in 5 to 10 parts of water, centrifuged, and decanted. This is repeated until the supernatant liquid is clear and colorless and gives no reduction test (6 to 7 washings). The yeast is then suspended in 10 parts of water. In this condition it will keep in a refrigerator for about two weeks.

Use of the Hagedorn-Jensen Method.—Two-tenths cubic centimeter of blood is collected into an accurately calibrated pipette (the authors use 0.2 ml. in 0.001 ml. Kahn pipette).

*At present the authors consider the 15- and 120-minute specimens unnecessary, and the 5-minute specimen relatively unimportant, except when the test is repeated on account of borderline results.

No. 37036), and transferred to 2.3 c.c. of distilled water, rinsing the pipette once or twice with the solution. One cubic centimeter of the 10 per cent yeast suspension is then added, the contents are mixed by tapping the tube against the hand, and after four or five minutes 0.5 c.c. of tungstic acid solution (prepared freshly by mixing equal volumes of 10 per cent sodium tungstate and $\frac{2}{3}$ N sulfuric acid) is added. The test tube is covered with the thumb, and the contents are mixed quickly by inverting. After standing a few minutes, the mixture is centrifuged at high speed. One cubic centimeter of the clear filtrate is used for titration.

Use of the Folin-Wu Method.—The yeast is washed in the usual manner and a 20 per cent yeast suspension is prepared. To 2 c.c. of oxalated blood 14 c.c. of the yeast suspension and 4 c.c. of the tungstic acid solution are added. This mixture is filtered, and 2 c.c. portions of the filtrate are used for sugar determinations.

The figure for nonfermentable reducing substances in the fasting blood is subtracted from the corresponding figure in the remaining specimens to obtain the galactose content of the blood titrated as glucose. In order to obtain the true value for galactose, which has a lower reducing power than glucose, 24 per cent must be added to the last figure.

Galactose is used in this test because, while having a rate of absorption very similar to that of glucose, it has the advantage that, unlike glucose, it can be identified in the blood.

HEMATURIA, Diagnostic Value of Occult, Barach, J. H., and Pennock, L. L. J. A. M. A. 114: 640, 1940.

Orthotolidine is a sensitive test for occult hematuria. A positive orthotolidine reaction was found in one-third of 681 general medical cases of the so-called chronic diseases. It is less common in youth, with more intact vessels, and it is more common in females than in males on account of the greater source of bleeding in the female generative tract. Seasonal variation pointed to the highest incidence during the summer months. Albuminuria and occult blood are found independently of each other, each having its own significance. Glycosuria is not a cause of occult bleeding. Arsenicals in therapeutic doses do not cause occult bleeding. Alkalinity or acidity of the urine is not a cause of occult bleeding. In patients showing persistent occult hematuria, hypochromic anemia is common. A review of the type of case in which the strongest reactions occurred reveals that these reactions were most pronounced in the clinically recognized serious types of disease and that the test reflects the patient's actual condition.

STREPTOCOCCI, Human Hemolytic From Diseases of Children, Boisvert, P. L. Am. J. Dis. Child. 59: 281, 1940.

The Lancefield group precipitin test gave consistent results and seems well adapted for epidemiologic use. The results suggest that infants and children are no more, and may be less, susceptible than adults to infection by hemolytic streptococci or groups other than A. It seems significant to the author that during a period of over a year all the strains of hemolytic streptococcal infections and their possible sequelae should belong in the one group, group A. Thus, 509 strains of group A hemolytic streptococci were consecutively obtained from 262 pediatric patients.

Infants and children are no more susceptible than adults to infection by hemolytic streptococci of groups other than A.

This study is being continued and is now in its fourth year. The only exception to date is the isolation this fall of a group F hemolytic streptococcus from the blood of a 7-year-old child with a nonfatal septicemia.

PERICARDIUM, Milk Spots, Nelson, A. A. Arch. Path. 29: 256, 1940.

Pericardial milk spots occurred in 170 (24.4 per cent) of 494 persons 1 year or more of age. In 439 persons 18 or more years of age the incidence was 37.6 per cent. In general, there is an increase of incidence with age, but this increase is by no means rectilinear. The spots are scarce in children and very frequent in old age, but between 35 and 75 years of age there is little change in incidence.

There seems to be a definite association with chronic or recurrent valvular heart disease; of 42 persons with such disease, 28 (66.7 per cent) showed spots. Patients with severe coronary sclerosis and enlarged hearts showed fairly definite increases (50.0 and 47.9 per cent, respectively).

The occurrence of more than one spot is slightly more frequent than that of only one. Spots occur on the right side, anteriorly, and on the ventricles much more frequently than they do on the opposite surfaces.

Old pleural adhesions are slightly more frequent in patients with spots than in those without.

Fifteen or 20 per cent of spots show appearances (projecting villi, cellular exudation, subepithelial, palisading, or epithelial enclosures in collagenous tissue) which suggest transitions from a more active inflammatory process to the usual type of milk spot.

SULFAPYRIDINE THERAPY, Nature of the Renal Lesion With, Stryker, W. A. J. A. M. A. 114: 953, 1940.

Precipitation of acetylsulfapyridine in the kidney was found at autopsy of a patient after treatment with sulfapyridine. The marked dilatation of cortical tubules and glomerular spaces, characteristic of kidneys in cases of sulfapyridine toxicity, seems to depend on intratubular precipitation of the drug or its compounds. There is a possible explanation for the previous difficulty in demonstrating the material in histologic sections.

DEXTROSE TOLERANCE, Tests of the New Born, Ketteringham, R. C. Am. J. Dis. Child. 59: 542, 1940.

By the Jeghers-Myers modification of the Folin-Mahros ferricyanide micromethod, the blood sugar values of 16 normal newborn infants, 5 to 10 days old, ranged from 70 to 95 mg. per hundred cubic centimeters three and one-half to four hours after feeding, with a mean of 81 mg.

Dextrose tolerance tests were made on 15 normal infants. Oral administration of 1.75 Gm. of dextrose per kilogram of body weight, as a 10 per cent solution, with blood sugar determinations one-half, one, two, three, and four hours after the midpoint of the period necessary for ingestion, produced an average maximum level of 145 mg. per hundred cubic centimeters. Two distinct curves were present; the blood sugar value of formula-fed infants reached a mean maximum of 143.2 mg. at thirty minutes, while that of breast-fed infants reached a mean maximum of 142.6 mg. at one hour.

Response to the dextrose meal was higher than in previously reported work; apparently these infants responded just as young adults do. The hypoglycemic reaction of Foster was encountered in 11 of the 14 babies tested.

Infants of diabetic mothers were more difficult to test than were the controls, and the curves produced seemed less reliable than those of the controls, owing to technical factors which could not be regulated. One infant who had previously showed the clinical signs of hypoglycemia had a strikingly erratic curve.

The Exton-Rose one hour, two-dose test, modified to the dose-weight principle, was administered to 3 normal infants, but the nature of the divided dose leads one to believe this technique unsuitable for the newborn.

GNORRHEA, Culture Method in the Diagnosis of, A New Medium, Pitts, A. C. Ven. Dis. Inform. 21: 67, 1940.

A relatively simple medium for the culturing of gonococci has been described.

This medium is a variation of the Schwartz-Davis medium and consists of a testicular infusion agar to which sterile hydrocele, ascitic, or pleural fluid is added. It has proved a most satisfactory medium for primary cultures.

The preparation of the medium follows:

Infuse 500 Gm. of minced sheep testicles in 1 liter of distilled water overnight in the icebox. (The testicles should be from recently killed animals.) Strain through gauze, boil vigorously for one-half hour, and strain through gauze again. Restore volume with distilled water and boil another one-half hour to complete coagulation, strain through gauze and

filter through paper. If coagulation is complete, filtration will be relatively easy by the use of suction; if coagulation is not complete, it is advisable to boil the infusion a little longer. Restore volume and add:

20 Gm. bacto-peptone
5 Gm. sodium chloride
5 Gm. dextrose

Adjust the reaction to pH 7.8 (pH should be between 7.4 and 7.6 after autoclaving) and add:

20 Gm. bacto-agar

Heat until agar is melted (autoclaving the mixture twenty minutes is a satisfactory method), filter through gauze and cotton, and tube approximately 5 c.c. per tube. Autoclave for thirty minutes at 10 pounds' pressure. When cooled to 50° C., add approximately 2 c.c. of sterile hydrocele fluid, rotate the tube to insure an even mixture, and slant. As soon as the agar is hardened, replace the cotton stoppers with rubber ones; it is important to do this as soon as possible in order to retain a maximum amount of moisture in the tubes. This medium will keep in the incubator for several months.

UREA, Rapid Methods for the Determination of, in Blood and Urine, Scott, L. D. Brit. J. Exper. Path. 21: 93, 1940.

1. Centrifuge tubes graduated at 5 c.c., and thick-walled test tubes of approximately 6 by $\frac{5}{8}$ inches.
2. Pipettes of 0.2 c.c., 1 c.c., 2.5 c.c., and 5 c.c. capacity.
3. Two 5 c.c. microburettes graduated to 0.05 c.c.
4. N/20 and N/100 sulfuric acid.
5. Approximately N/50 sodium hydroxide.
6. No. 1 Whatman filter papers of 5.5 cm. diameter.
7. Absolute alcohol and redistilled or "Analar" acetone.
8. Special mixed indicator:

Stock Solution A: 0.02 per cent methyl red in 60 per cent (by volume) absolute alcohol. The solid methyl red should be ground up with absolute alcohol in a small mortar till completely dissolved, and transferred to the 100 c.c. flask by repeated washings with absolute alcohol in the usual manner. Forty cubic centimeters of water are added and the volume is brought to 100 c.c. with absolute alcohol.

Stock Solution B: 0.10 per cent aqueous methylene blue.

The indicator is prepared by adding 1 c.c. of the methylene blue (solution B) to 15 c.c. of methyl red (solution A). The mixture, if green in color, is brought to a very faintly acid reaction by adding N/10 hydrochloric acid drop by drop with constant shaking until the solution just turns reddish purple. This solution keeps indefinitely if stored in an amber ground glass-stoppered bottle. It is reddish purple in acid solution, and bright green in alkaline solutions.

9. Neutral "Urease-Dunning" enzyme suspension:

Four 25 mg. tablets of "Urease-Dunning" are thoroughly ground up in a small mortar with 4.5 c.c. of N/100 sulfuric acid. When an even emulsion has been obtained, 0.2 c.c. of the mixed indicator is added. The extract, if correctly prepared, has a grayish buff coloration (the neutral point of the indicator) and keeps in the refrigerator for one month.

Determination of Urea in Blood.—Into one of the 5 c.c. graduated centrifuge tubes, containing 3 to 4 c.c. of absolute alcohol or acetone, pipette 1 c.c. of serum, oxalated whole blood, or plasma. As it is impossible to remove the last traces of blood from the pipette by sucking up and blowing out with alcohol in the usual manner (due to the pipette becoming clogged with precipitated blood protein), the pipette must be allowed to drain thoroughly for about one minute, and the last traces of blood removed by expelling the air. By this procedure not more than 0.01 c.c. of blood is retained in the pipette. The centrifuge tube is now securely stoppered and vigorously shaken, after which the volume is readjusted to 5 c.c. by the further addition of a few drops of absolute alcohol or acetone, as the case may be, to counteract the reduction in volume due to the formation of alcoholic hydrates. Mix again by shaking well and centrifuge at high speed for two to three minutes, or filter

through a No. 1, 5.5 cm. paper. After filtration the precipitated blood protein contained in the filter paper should be disturbed by very gentle stirring with a small glass rod, otherwise the 2.5 c.c. of filtrate may not always be obtained from bloods rich in metaprotein. Measure 2.5 c.c. of supernatant fluid or filtrate into one of the thick-walled test tubes and add 3.5 c.c. of water. The alcoholic extract becomes slightly opalescent due to the partial precipitation of blood fat by the lowered alcoholic concentration. Three-tenths cubic centimeter of special indicator is added and the alkaline reaction (green) brought over to the acid side (reddish purple) by the addition of N/20 sulfuric acid, with thorough mixing after the addition of each drop. The alcoholic extract varies in alkalinity according to the blood bicarbonate content. Fiftieth-normal sodium hydroxide is carefully added until the reaction is just alkaline again, and the reaction finally brought over to the very faintly acid point of the indicator by the addition of N/100 sulfuric acid, drop by drop, with constant shaking. By this readjustment of the pH, final titration with relatively strong acid is avoided, enabling the starting point of the reaction to be controlled to within one drop of N/100 sulfuric acid, which in terms of blood urea is approximately 1 mg. per 100 c.c. Finally, 0.35 c.c. of well-shaken neutral urease suspension is added, and after thorough mixing, the tube is securely stoppered and heated in the water bath at 50° to 55° C. for ten to fifteen minutes. Remove the tube from the bath and titrate with N/100 sulfuric acid until slightly acid (the original reddish purple starting point of the reaction). The titration value in cubic centimeters multiplied by 60 equals milligrams of urea per 100 c.c. of blood.

Determination of Urea in Urine.—For the estimation of urea in urine a 1:50 dilution of the specimen is made with distilled water (with small quantities of urine 0.2 c.c. can be measured with a blood pipette into 9.8 c.c. of water) and after mixing well, 5 c.c. is pipetted into a thick-walled test tube and 0.3 c.c. of indicator is added. The faint greenish coloration obtained with the majority of urines is due to the urine being alkaline to the special indicator. Adjustment is made to the faintly acid side of the indicator by the addition of N/100 sulfuric acid drop by drop. One-half cubic centimeter of well-shaken neutral urease suspension is now added, the tube is securely stoppered, and the mixture (which turns green almost immediately) is heated in the water bath at 50° to 55° C. for five minutes. The ammonium carbonate so formed is back titrated with N/20 sulfuric acid till just reddish purple, i.e., the original starting point of the reaction. The titration value in cubic centimeters multiplied by 1.5 equals grams of urea per 100 c.c. of urine.

BLOOD, A Familial Hematopoietic Disorder in Italian Adolescents and Adults, Wintrobe, M. M., Matthews, E., Pollack, R., and Dobyns, B. M. J. A. M. A. 114: 1530, 1940.

Fourteen individuals, members of three Italian families representing three generations in one family and two in another, have been examined and have been found to have certain abnormalities.

In four marked poikilocytosis, microcytosis, hypochromia, and stippling of the red blood cells as well as corpuscles resembling targets were observed. In addition, there were increased resistance of the red corpuscles to the hemolytic action of hypotonic saline solutions, splenomegaly, and bilirubinemia or urobilinuria. One of these patients also had erythroblastosis in the sternal bone marrow and slight osteoporosis.

In four persons all the abnormalities mentioned except splenomegaly were found. In two children there was slight splenic enlargement without significant changes in the blood. Of the remaining four persons, all females, there was microcytosis without other significant changes in two, and slight macrocytosis as well as stippling in two.

This disorder may be considered to be a benign form of Cooley's anemia or Mediterranean disease.

This condition and Cooley's anemia appear to be the result of an inherited defect in the production of red corpuscles whereby corpuscles are formed with an adequate or excessive membrane which contains little substance. As a result the cells can withstand the hemolyzing effect of hypotonic saline solutions more readily than normal cells. The other characteristics of the disorder are explained by the assumption that they are due to attempts to compensate for the faulty red blood cell formation.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor,
Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

The Fundamentals of Internal Medicine*

DESIGNED primarily for the introduction of students to the study of internal medicine, this volume, nevertheless, contains good chapters dealing with otolaryngology, ophthalmology, and dermatology. Because photographs of dermatoses show up better in white persons than in Negroes, it is felt that the section on dermatology would be improved by changing such illustrations. A chapter on dietetics is also provided.

The work is intended to provide the foundation upon which the superstructure of more detailed and extensive knowledge may be built. In this aim it succeeds admirably.

Cancer†

THIS volume was prepared by a committee representing the Massachusetts Medical Society and the American Society for the Control of Cancer. It is intended for the general practitioner and is designed to aid him in the diagnosis of early cancer and to suggest the treatment recognized at the present time. Chapters on cancer of the various organs are written by men especially interested in the subject.

The discussion of the care of the patient with advanced cancer, and neurologic methods of relief of pain are also handled in an able manner.

The various organizations for the control of cancer, local and national, are described. There is a concluding chapter on suggestions for talks on cancer to lay audiences.

The book is brief and is not intended to take the place of the more extensive treatises in the field.

It is felt that this is excellent propaganda, using the word in its best sense.

Manson's Tropical Diseases‡

PROBABLY the outstanding text in its field, this book has now come into the eleventh edition. Although the term "tropical medicine" is in general usage, it is perhaps a misnomer. One has only to recall the Chicago outbreak of amebiasis, the cases of malaria in

Medicine. By Wallace Mason Yater, A.B., M.D., M.S. and Director of the Department of Medicine, Georgetown University Hospital; Physician-in-Chief, Georgetown University Hospital; Washington, D. C. Formerly Fellow in Medicine, the Johns Hopkins Hospital. D. Appleton-Century Company, Inc. Cloth, 1,021 pages.

†Cancer—A Manual For Practitioners. The Committee on Publication: George W. Holmes, M.D., Chairman; Ernest M. Daland, M.D.; Shields Warren, M.D.; and Channing C. Simmons, M.D., Editor. Cloth, 284 pages. Boston, Mass., 1940.

‡Manson's Tropical Diseases. A Manual of the Diseases of Warm Climates. Edited by Philip H. Manson-Bahr, C.M.G., D.S.O., M.A., M.D., D.T.M.; and H. Cantab, F.R.C.P. London, Senior Physician to the Hospital for Tropical Diseases, London, the Albert Dock Hospital and the Tilbury Hospital; and the Colonial Office and Crown Agents for the Colonies; and the Royal Air Force; Director, Division of Clinical Medicine, Tropical Medicine to the I. North-Eastern Post-Graduate School of Medicine; Member of the Council of the Conjoint Board of the Royal College of Physicians, London, and to Cambridge and Work of Sir Patrick Manson, 1927; and "The Dysenteric Disorders," 1930. Cloth, ed. 11, revised, 1,043 pages, 18 color plates, 15 halftone plates, 364 illustrations in text, 6 maps, and 28 charts, \$11.00. A William Wood & Co. book. Williams & Wilkins Co., Baltimore, Md., 1940.

narcotic addicts, the widespread incidence of intestinal parasites, and the great amount of bacillary dysentery, to realize that we have many "tropical" diseases in this country. In addition to a standard description of these and many other diseases, the book contains a section on life in the tropics of great value to anyone contemplating a trip or an extended sojourn into tropical climates. Anyone called upon to give advice to such persons will not be disappointed if he seeks information at this source.

Clinical Diabetes Mellitus and Hyperinsulinism*

IN THIS easily readable book the author has limited himself to clinical considerations describing procedure which in his extensive experience has proved most effective in the recognition and treatment of diabetes mellitus and its complications. Extended consideration of the theory of carbohydrate metabolism, the physiology of experimental diabetes, and the pathology of diabetes are omitted.

Description of techniques of diagnosis, diet, and insulin (old and new) is given in sufficient, but not excessive, detail. Much attention is directed toward the ills which so often are concomitant. Chapters are devoted to surgical operations and to pregnancy in diabetes.

A thorough section is afforded to hyperinsulinism in the last part of the book. There is a good bibliography and index.

Principles of Hematology†

IT IS pointed out that the symptoms of blood disorders do not help greatly in differentiation. An anemia from any cause will result in weakness, pallor, and palpitation of the heart. There may be fever, anorexia, dizziness, and edema. Few of the anemias have characteristic accompanying symptoms, with the striking exception of pernicious anemia. There are few characteristic symptoms of leucemia. The disease may be suspected from a splenomegaly, enlarged glands, fever, unexplained anemia, or abnormal bleeding. A careful history may reveal the explanation of an anemia, such as a previous blood loss or an acute or chronic infection. A thorough physical examination may reveal a malignancy.

Written by an outstanding hematologist, the book is well balanced. The anatomy and physiology of the blood, the mechanisms involved in the various dyscrasias, the techniques of blood examination, and finally treatment, are all excellently integrated.

*Clinical Diabetes Mellitus and Hyperinsulinism. By Russell M. Wilder, M.D., Ph.D., F.A.C.P., Professor and Chief of the Department of Medicine, the Mayo Foundation for Medical Education and Research, University of Minnesota; Head of the Section on Metabolism Therapy, Division of Medicine, the Mayo Clinic, Rochester, Minn. Cloth, 450 pages, illustrated. W. B. Saunders Co. Philadelphia and London, 1940.

†Principles of Hematology. By Russell L. Haden, M.A., M.D., Chief of the Medical Division of the Cleveland Clinic, Cleveland, Ohio; Professor of Experimental Medicine in the University of Kansas School of Medicine. Cloth, ed. 2, thoroughly revised, 362 pages, with 104 illustrative cases including 173 original photomicrographs and 100 original charts and drawings. Lea & Febiger, Philadelphia, 1940.

EDITORIAL

Clinical Chemistry

A NEW section on Clinical Chemistry is being inaugurated with this issue of the JOURNAL. This does not mean that the original scope of the JOURNAL, as defined by its first Editor, Dr. Victor C. Vaughan,¹ in the first issue, has in any way been changed. In the quarter of a century that has elapsed, many important advances in medicine have been made, and chemistry has contributed a large number of them. This has resulted in a considerable increase in the number of papers which have been devoted to studies falling under the classification of clinical chemistry. For this reason, it now seems desirable to group these articles together under this special heading.

Dr. Vaughan unquestionably visualized the expansion in this field when he founded the JOURNAL, since he was a pioneer* in this country in the field of biochemistry and clinical chemistry—then called physiological chemistry and pathological chemistry, respectively. It is pertinent to note that the subject of blood chemistry was in its infancy in 1915 when this JOURNAL was founded. The expansion which has taken place in this field is typical of the expansion which has occurred in many other fields of biochemistry. Although the accomplishments in blood chemistry are probably no more spectacular or important than those which have taken place in the field of the vitamins or of the hormones, still blood chemistry requires much greater use of the clinical laboratory and has more far-reaching ramifications. The latter is well illustrated by the fact that the action of vitamin D is shown by studies of the inorganic phosphorus and calcium of the blood serum, while that of insulin requires the determination of the blood sugar.

Recently the methods of chemical blood analysis have been extended to tissue analysis; this will doubtless greatly expand the interpretations which can be made upon the basis of blood analysis alone. By utilizing some of these micromethods after still further refining them, Richards,² in particular, has been able to develop a sound theory of urinary secretion, thus placing our chemical tests of renal function upon a firmer foundation. Illustrative contributions in the field of clinical chemistry need not be further multiplied.

While this JOURNAL has had a hand in presenting some of the most important original contributions—for example, Banting and Best³ presented their initial report on insulin here, and Stanley Benedict⁴ reported the excretion of uric acid by the Dalmatian coach dog, thus permitting the extension of studies on purine metabolism previously restricted to man (and the anthropoid apes)—still its greatest service to medicine has been in bringing new practical tests to the clinical laboratory and in turn to the clinic. This is in accord with the title of the JOURNAL and Dr. Vaughan's first editorial,¹ which indicated that the

*Dr. Vaughan was appointed Instructor in Physiological Chemistry at the University of Michigan in 1876.

purpose of the JOURNAL was to try to bridge the gap between the laboratory worker and the clinic, so that the physician could intelligently utilize without unnecessary delay the new laboratory aids which were rapidly becoming available. This need is even greater today than it was twenty-five years ago.

Clinical chemistry may be defined as that part of biochemistry which finds use in the diagnosis, prognosis, and clinical management of disease. The JOURNAL will accept for the section on clinical chemistry original articles, including methods, which meet a reasonably broad interpretation of this specification. It is hoped that this new section will be found increasingly useful by internists and clinical chemists alike.

V. C. M.

E. M.

REFERENCES

1. Vaughan, V. C.: The New Journal, J. LAB. & CLIN. MED. 1: 57, 1915.
2. Richards, A. N.: Physiology of the Kidney, Bull. New York Acad. Med. 14: 5, 1938.
3. Banting, F. G., and Best, C. H.: The Internal Secretion of the Pancreas, J. LAB. & CLIN. MED. 7: 251, 1922.
4. Benedict, S. R.: Uric Acid in Its Relation to Metabolism, J. LAB. & CLIN. MED. 2: 1, 1916.

Erratum

On page 1301 of the September number of the JOURNAL in the article by Ida Kraus, Ph.D., entitled "A Note on the Determination of Total Serum Proteins, Serum Albumin, and Serum Globulin," c under *Calculations* should read:

$$\text{N.P.N.} : \frac{S}{U} \times \text{Conc. standard} \times \frac{15}{4.5} \times \frac{100}{1} = \text{N.P.N.} / 100 \text{ c.c. serum.}$$

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PROGRESS

A CRITICAL HISTOPATHOLOGIC STUDY*

FIFTY POST-MORTEM PATIENTS WITH CANCER SUBJECTED TO LOCAL OR
GENERALIZED REFRIGERATION COMPARED TO A SIMILAR CONTROL
GROUP OF 37 NONREFRIGERATED PATIENTS

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IN PREVIOUS reports¹ the several members of our group who have been working upon the problems of reduced temperatures as applied particularly to cancer and embryonal cell growth have recorded their preliminary observations and deductions in rather broad generalities. This has been done intentionally in order to arouse interest in hypothermy both as a possible therapeutic agent and as a new approach experimentally to our understanding of human physiology.

The purpose of this paper is to reinforce these earlier studies by a more detailed analysis of the pathologic material obtained from the first 50 cancer patients who had undergone varying periods of refrigeration, either local, general, or combined, and who subsequently died. As a control, a similar series of 37 patients with cancer who died without having received refrigeration has been studied comparably. In this latter group the only selective factor which has entered the picture has been an effort to choose persons with the same primary organ involvement as those in the refrigeration series.

As it works out the refrigeration group tended to include, as might be expected, more advanced cases. The autopsies were all done by the same staff members, so that the two groups are strictly comparable in respect to the interpretation of any abnormal findings. We are not concerned in this paper

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with any of the clinical aspects of the cases other than statistical; i.e., age of patient, duration and extent of disease, location of primary tumor, former treatment, whether surgical, radiologic, or combined, and the time relationship of such treatments, recurrence, metastases, etc. From the standpoint of refrigeration, ideally we should like to collect sufficient data to be able to tell whether continuous application is of greater effect than when refrigeration is given on the divided or fractionated dose principle, whether it is the *total* number of hours or the depth to which the temperature is successfully lowered and maintained, or some combination of these various procedures, which is the most successful in producing regressive cell changes in tumor tissue. As it is, in a small series of this size the most we can hope to do is to divide the cases on the basis of total number of hours of refrigeration received.

Since the publication of our initial studies concerning the effect of cold upon cancer and embryonal cell growth, a very considerable amount of discussion has resulted. One of the more critical of the statements has intimated that the growth of cancer cells is not only *not* impaired by cold, but is even *accelerated* and results in a wider dissemination of metastases. True, the observations are based on animal experiments² rather than on clinical studies. However, we feel that it is most important to know whether such acceleration of growth does occur in human beings or to what degree it is indicated, as upon such information depends the clinical selection and conduct of our cases. It is to study such questions that the careful and critical analysis of these autopsied cases seems so important to us, groping as we are in a field which is as yet totally unexplored.

Even though time may prove that such acceleration of cell growth may occur ultimately with refrigeration, it will not exclude refrigeration as a possible therapeutic weapon any more than it will exclude x-ray or surgery in which such effects are well recognized as occurring at times. The most pessimistic among us cannot deny that hypothermy has helped solve the problem of intractable pain in our patients with cancer. Any therapeutic agent must be weighed seriously as to its relative advantages and disadvantages before it is either used or discarded. It is from this purely objective standpoint that we wish to present in tabular form the histopathologic findings in these initial 50 refrigeration patients who were autopsied and compare them with a similar nonrefrigerated series.

The patients fell into three major groups, and a fourth group made up of miscellaneous forms of cancer. The three principal groups were (1) mammary, (2) uterine-cervical, and (3) gastrointestinal carcinoma. The fourth group was composed of tumors from almost every other part of the body, including lung, liver, adrenal, bladder, prostate, testis, mouth, thyroid, bone, lymph nodes and brain, as well as two cases of acute leucemia.

Fifteen of the 50 patients received local refrigeration treatment only, and properly might be excluded from the series as a whole. The great majority of the patients received less than 150 hours of generalized refrigeration (i. e., 35 persons, or 60 per cent), the time ranging from 24 to 150 hours. Only 5 persons received more than 150 hours of general hypothermy in this earlier series, although in subsequent studies more than twice this time interval was

not infrequently employed. It should be noted also, that in this initial series of cases the temperature level in general was maintained between 86° and 90° F., whereas in the later cases temperatures of approximately 8° to 10° lower were employed. Thus, the further analysis of a larger series of cases studied in the lower temperature range and maintained at these lower levels for much longer periods of time may require the revision of our figures and lead to other interpretations than are possible with these data.

TABLE I
MAMMARY CANCER—24 PATIENTS

ORGAN	LESION	REFRIGERATION GROUP		NONREFRIGERATION GROUP		HENKE AND LUBARSCH (%)	EWING 423 PATIENTS (%)
		NO. PATIENTS	%	NO. PATIENTS	%		
Heart	Normal	1	8.3	1	8.3	5	
	Myocardosis I and II	8	66.6	4	33.3		
	Myocardosis III and IV	1	8.3	7	58.1		
	Myocardial edema	2	16.6	3	25.0		
	Metastases	2	16.6	3	25.0		
	Pericarditis	1	8.3	0	0		
Lung	Bronchopneumonia	7	58.3	4	33.3	50	49.9
	Metastases	12	100.0	9	75.0		
Liver	Degenerations	7	58.1	5	41.5	48-63	48.6
	Metastases	8	66.6	8	66.6		
Spleen	Metastases	0	0	1	8.3	19	7
Kidneys	Toxic nephrosis	4	33.3	2	16.6	5-10	
	Metastases	2	16.6	2	16.6		
Pancreas	Pancreatitis	1	8.3	0	0	Rare	
	Metastases	1	8.3	1	8.3		
Adrenals	Medullolosis	2	16.6	1	8.3	27-56	
	Metastases	4	33.3	5	41.5		
O. I. tract	Metastases	0	0	1	8.3	Rare	
Uterus	Metastases	2	16.6	2	16.6	5	
Ovary	Metastases	5	41.5	None noted grossly		8	
	Metastases	2	16.6	None noted grossly		Rare	
Thyroid	Metastases	1	8.3	None noted grossly		Rare	
Pituitary	Metastases	12	100.0	Only 4 cases recorded but examination incomplete		19-80	20
Skeleton	Vertebral metastases	2	16.6	None noted clinically		3-19	9.5
	Other bones	4	33.3	None examined			
Brain and dura	Metastases	3	25.0				
	Edema						

With these preliminary comments we will present the statistical data in tabular form, comparatively arranged in the several groups for convenience. In Table I 24 cases of advanced breast cancer are shown. These were chiefly of the "simplex" type and varied from extremely cellular, medullary lesions to equally extreme scirrhous cases. The age of the patients ranged from 32 to 56 years, those in the lower age group predominating. Of the 12 patients subjected to refrigeration, all but one received generalized hypothermy, this one received cold locally. The 12 control patients received the usual suppor-

tive and symptomatic treatment. Several instances of operative and radiologic intervention are included in this latter group. Each of the persons who was refrigerated came to the hospital because of intractable pain, after all possible surgical and irradiation therapy had been employed, and after opiates had become ineffective. The duration of the generalized refrigeration varied from 50 to 230 hours, and the temperature range was from 83° to 90° F, the average being about 87° F.

Table I gives us a certain amount of information, much of it negative in character, but nevertheless of some importance and significance in our efforts to evaluate the effect of reduced body temperatures upon the activity of cancerous cells. In the first place, it is obvious that the entire 24 patients represent a much more advanced stage of malignancy than the average admission group, as shown by the widespread metastases in comparison with the figures from world literature compiled by Henke and Lubarsch in their *Handbook of Pathology*, and the series of 423 cases of breast cancer analyzed by Ewing. The metastatic figure is even greater in the 12 persons given refrigeration than in the corresponding 12 control patients, showing 100 per cent lung and skeleton involvement as compared with 75 and 33 per cent, respectively. Furthermore, these metastases were all present at the time of admission. Comment upon their serial roentgen-ray studies will be made later in this discussion in relation to the entire 50 cases. Suffice it to say that from the data available, we have no evidence to suggest that the rate of growth of such metastatic foci is accelerated. On the contrary, in some few instances it actually seems retarded. However, in view of the advanced stage of the disease in this group of patients as a whole, it is not possible either to prove or to disprove whether hibernation does or does not increase the spread of metastases or even alter the rate of growth of such metastatic tissue within the relatively short span of life during which they were under observation.

TABLE II

RELATIONSHIP OF METASTASES (PULMONARY) TO DEVELOPMENT OF BRONCHOPNEUMONIA IN 24 PATIENTS WITH BREAST CANCER

GROUP	PATIENTS WITH METASTASES					PATIENTS WITH NO METASTASES				
	NO. WITH METASTASES	BRONCHOPNEUMONIA		NO PNEUMONIA		NO. WITHOUT METASTASES	BRONCHOPNEUMONIA		NO PNEUMONIA	
		NO.	%	NO.	%		NO.	%	NO.	%
Refrigerated	11	7	58.3	5	41.7	0	0	0	0	0
Nonrefrigerated	9	3	33.3	6	67.0	3	1	33.3	2	67

Aside from the question of metastases two other features of interest pathologically have been noted which relate (1) to the part played by the heart during refrigeration and (2) the incidence and importance of bronchopneumonia comparatively in the refrigerated and nonrefrigerated cases. Both of these questions will be discussed in greater detail later, in respect to the entire 87 cases. So far as myocardial failure is concerned there seems to be no significant difference between the two groups.

The incidence of bronchopneumonia requires more detailed analysis. There is one factor which seems possibly significant in influencing the occurrence of pneumonia in both the refrigerated and nonrefrigerated groups, viz., the

presence or absence of metastases. The series of patients with cancerous breast seem to be representative of this point, having a particularly high incidence of metastases because of their anatomic relationship. In Table II the data are presented with this in mind, and an attempt is made to correct statistically for the difference in pulmonary metastatic incidence in the two groups.

From these figures it can be stated that the presence of pulmonary metastases, as might well be anticipated, is apparently associated with a somewhat higher incidence of terminal bronchopneumonia in the refrigerated series of persons with breast cancer. It might be added that under such metastatic conditions, the pneumonic incidence appears to be somewhat greater in the cases maintained under generalized refrigeration for the more prolonged periods of time. In a series of cases as small as this we are not certain whether the differences are of such magnitude as to be statistically significant, but we have gained the impression that where such pulmonary metastases do exist one must recognize an added risk in recommending generalized refrigeration for any given case.

TABLE III
CERVICAL CANCER—10 PATIENTS

ORGAN	LESION	REFRIGERATION GROUP		NONREFRIGERATION GROUP		HENKE AND LUBAUSCH (%)	OTHER AUTHORS (%)
		NO. PATIENTS	%	NO. PATIENTS	%		
Heart	Normal	1	20	0	0		
	Myocardosis I and II	4	80	4	80		
	Myocardosis III and IV	0	0	1	20		
	Metastasis	0	0	0	0		
Lung	Bronchopneumonia	2	40	3	60		
Liver	Metastasis	1	20	1	20		5
	Toxic changes	4	80	2	40	15	
Spleen	Metastasis	0	0	2	40		
	Toxic changes	4	80	2	40		
Kidney	Metastasis	0	0	0	0		
	Pyonephrosis	1	20	1	20		
Bladder	Toxic nephrosis	3	60	2	40		
	Metastases	0	0	0	0		
Pancreas	Metastatic extension	2	40	1	20		
Adrenal	Pancreatitis	1	20	0	0		
	Metastasis	0	0	1	20		
Intestine	Metastasis	0	0	1	20		
Uterus	Metastasis	1	20	0	0		
	Metastatic extension	2	40	4	80		14-50 (Sheib, Schottlander)
Ovary	Metastasis	0	0	0	0	Rare	
Brain	Metastasis	0	0	No post-mortem examination made			
	Edema	2	40				
Skeleton	Metastasis	0	0	0	0		

The second group of patients, somewhat smaller in number, who had advanced squamous carcinoma of the cervix uteri, are analyzed in Table III in the same way as the patients with cancer of the breast were presented. Of

the five patients who received refrigeration, only three were given cold locally, while the other two required generalized refrigeration to control the pain. As might be expected, the incidence of metastases in this group of cases was much less than in the breast cases, and inasmuch as cold could be applied successfully locally at a much lower temperature range than that obtained by the generalized reduction of body temperature, it was the method of choice.

A series of ten patients with cervical carcinoma, analyzed in Table III, presents a totally different clinical problem than the patients with breast cancer. The disease is a local one, with regional extension of the tumor rather than distant metastasis. In only two cases were pulmonary metastases found, and in neither of these was pneumonia present terminally.

The third group, consisting of twelve persons with carcinoma of gastrointestinal tract, do not parallel each other quite as closely as the previous series, there being a slight preponderance of gastric cases in the nonrefrigerated series. Of the remaining five experimental cases, two were primary in the transverse colon, one in the sigmoid, one in the rectum, and one represented a peptic ulcer just below the pyloric ring which had undergone malignant degeneration and showed widespread metastasis. The analysis of this series is presented in Table IV.

TABLE IV
GASTROINTESTINAL TRACT CARCINOMA—12 PATIENTS

ORGAN	LESION	REFRIGERATION GROUP		NON-REFRIGERATION GROUP		HENKE AND LUBARSKY (%)	OTHER AUTHORS (%)
		NO. PATIENTS	%	NO. PATIENTS	%		
Heart	Normal	0	0	0	0	0.5	
	Myocardosis I and II	3	50	5	83.5		
	Myocardosis III and IV	3	50	1	16.5		
	Metastasis	0	0	0	0		
Lung	Bronchopneumonia	4	66.6	3	50.0	4-13	
	Lobar pneumonia	0	0	1	16.5		
	Empyema	1	16.5	0	0		
	Metastasis	2	33.3	2	33.3		
Liver	Toxic changes	3	50	1	16.5	45-56	34
	Metastasis	4	66.6	2	33.3		
Spleen	Toxic changes	1	16.5	0	0	3.4-6.5	8.3
	Metastasis	1	16.5	0	0		
Kidneys	Toxic changes	1	16.5	2	33.3	1.3-4.4	13
	Pyonephrosis	1	16.5	0	0		
	Metastasis	1	16.5	0	0		
Bladder	Metastatic invasion	1	16.5	0	0	1.1	
Pancreas	Pancreatitis	1	16.5	0	0	3.3	
	Metastasis	2	33.3	0	0		
Adrenals	Adenoma	2	33.3	0	0	1.3-7.6	6
	Metastasis	2	33.3	0	0		
Uterus	Metastatic invasion	1	16.5	0	0	0.5	
Ovaries	Metastasis	0	0	0	0		
Prostate	Metastasis	1	16.5	0	0	0.1	
Thymus	Persistence	1	16.5	0	0		
Brain	Edema	1	16.5	0	0		
	Metastasis	0	0	0	0		
Skeleton	Metastasis	0	0	0	0		

In this group of gastrointestinal cases of malignancy, all the experimental cases received both local and general refrigeration. The time of the latter ranged from 150 to 230 hours, a distinctly more prolonged technique on the average than that used in the breast group. Yet when we look at the figures relating to the incidence of bronchopneumonia in these cases, there is no significant difference between the refrigerated and the nonrefrigerated cases. Even the presence of lung metastases does not seem to be an unduly important factor in determining this complication, as less than half the cases in the series of cervical and gastrointestinal carcinoma with lung metastases demonstrated any evidence of bronchopneumonia at autopsy.

Another point to which attention should be called is the curious finding of occasional cases of acute pancreatic degeneration microscopically. This may be accompanied by a small amount of fat necrosis and hemorrhage, and thus become a grossly demonstrable process in rare instances, as has been observed in more recent cases than those subjected to analysis in this early series. We are inclined to feel that pancreatitis is another possible complication of generalized refrigeration. On the basis of Oppenheimer and McCravery's³ studies, which established the fact that circulation time is definitely slowed, this can be explained perhaps as being the result of tissue anoxia, thus permitting the escape and activation of the pancreatic enzymes. We are of the opinion, however, that careful following of the blood amylase of patients during "hibernation" precludes serious danger.

TABLE V

27 PATIENTS WITH MISCELLANEOUS MALIGNANCY SUBJECTED TO REFRIGERATION

CASE NO.	SITE OF PRIMARY TUMOR	DIAGNOSIS	REFRIGERATION PERIOD	
			GENERAL (HR.)	LOCAL
1	Adrenal	Hypernephroma, malignant	-	5 weeks
2	Antrum	Carcinoma, squamous cell	-	12 weeks
3	Antrum	Carcinoma, squamous cell	-	10 days
4	Auditory canal	Carcinoma, squamous cell	-	3 weeks
5	Bladder	Carcinoma, squamous cell	48	12 weeks
6	Bladder	Carcinoma, squamous cell	-	16 weeks
7	Blood	Leucemia, acute lymphatic	90	-
8	Blood	Leucemia, acute lymphatic	230	-
9	Bone (tibia)	Osteogenic sarcoma	90	-
10	Bone (sacrum)	Fibromyxosarcoma	-	200 days
11	Brain	Glioblastoma multiforme	-	3 weeks
12	Liver	Hepatoma malignant	90	-
13	Lung	Bronchiogenic carcinoma	24	3 days
14	Lung	Bronchiogenic carcinoma	90	1 day
15	Lymph node	Hodgkin's disease	120	-
16	Mediastinum	Lymphosarcoma	50	-
17	Pancreas	Adenocarcinoma—duct type	50	-
18	Prostate	Adenocarcinoma	-	6 weeks
19	Retroperitoneum	Fibromyxosarcoma	120	-
20	Retroperitoneum	Reticulo-cell sarcoma	24	-
21	Skin	Melanoma, amelanotic, malignant	120	-
22	Submaxillary	Adenocarcinoma	-	30 weeks
23	Testis	Choriocarcinoma	-	1 week
24	Thyroid	Adenocarcinoma	120	-
25	Tongue	Carcinoma, squamous cell	50	-
26	Tonsil	Carcinoma, squamous cell	24	-
27	Uterus	Adenocarcinoma—fundus	-	3 weeks

In presenting the remaining 27 cases, the data have been condensed so far as possible into comparable tabular form as in the previous groups. The cases, however, as shown in Table V, are almost entirely single instances of a very wide variety of neoplasms, selected in part on that very basis in order to have the opportunity of studying the effect of reduced temperatures upon as many types of malignant cells as possible. For that reason, it is obviously impossible to present statistical data in respect to any one of these various forms of malignant disease individually, but the composite figures appear to fit in with those of the three other series already presented.

DISCUSSION

At this point it seems well to recapitulate the major pathologic findings which this survey has disclosed, and to try to compare them in the refrigerated and nonrefrigerated series of patients. As has been commented upon in earlier papers, the persons who have been observed under refrigeration in this study were all advanced, terminal examples of malignancy. None of them, therefore, have had a survival period long enough to be of much significance in respect to the question of the effect of cold upon metastatic foci. The interest centers chiefly upon the immediate effects of cold on neoplastic cell growth and on various physiologic mechanisms, as evidenced by demonstrable histopathologic changes in the tissues of the several organ systems. To this end let us attempt to discuss the findings separately in those organs where such demonstrable pathology has been found to exist.

Heart.—As has been shown by others,⁴ as well as by our own studies,^{5,6} generalized reduction of body temperature is quite regularly accompanied by alterations in the electrocardiogram which suggest rather widespread myocardial involvement; i.e., a depression of the T-wave and prolongation of the Q-S interval.

Furthermore, occasional instances of sudden fall in blood pressure followed by heart failure, and associated with very acute myocardial degeneration at autopsy, led us to suspect that the slowed circulation might present a serious problem insofar as maintenance of the nutrition of the heart muscle was concerned. Analysis of the autopsy material in these cases under consideration has been somewhat reassuring in this respect. In the 50 patients subjected to refrigeration a total of 42 (84 per cent) have shown some degree of myocardial degeneration, by far the greater number of grades I and II. On the other hand, 35 (94 per cent) of the 37 control patients have shown similar changes, with a definitely higher proportion in the grades III and IV classification comparatively.

It is doubtful whether any real importance should be placed on these relatively minor differences of percentage, although the grading in each instance was done by three members of the department independently with only very few and minor discrepancies. Thus, it seems fair to state that there is no apparent or significant difference in the actual incidence of myocardial degeneration in the two groups.

Respiratory Tract.—In attempting to evaluate the data relating to the respiratory tract, one problem, that of the incidence and relationship of broncho-

pneumonia to refrigeration, is obviously paramount. We have already seen in presenting the figures from the group of patients with breast cancer that the existence of pulmonary metastases seemed to increase the likelihood of the development of a bronchopneumonia during generalized refrigeration. However, in the comparable series of cases of breast cancer in the nonrefrigerated group the percentage incidence of terminal bronchopneumonia was identical (67 per cent) in the metastatic and nonmetastatic cases.

TABLE VI

COMPARATIVE FINDINGS (A) IN 41 PATIENTS WITH MISCELLANEOUS MALIGNANCY AND (B) IN ENTIRE COMBINED GROUPS—91 PATIENTS

ORGAN	LESION	(A)				(B)			
		REFRIGERATED GROUP (27 PATIENTS)		NON-REFRIGERATED GROUP (14 PATIENTS)		REFRIGERATED GROUP (50 PATIENTS)		NON-REFRIGERATED GROUP (37 PATIENTS)	
		NO. PATIENTS	%	NO. PATIENTS	%	NO. PATIENTS	%	NO. PATIENTS	%
Heart	Normal	4	15.0	1	7.1	6	12.0	2	5.4
	Myocardosis I and II	19	70.0	10	71.4	34	68.0	23	62.1
	Myocardosis III and IV	4	15.0	3	21.4	8	16.0	12	32.4
Lungs	Metastasis	5	18.5	3	21.4	5	10.0	6	16.2
	Bronchopneumonia	18	66.6	9	64.2	30	60.0	19	51.3
	Lobar pneumonia	1	3.0	0	—	1	2.0	1	2.7
Liver	Metastasis	9	33.3	5	35.7	24	48.0	17	45.9
	Toxic changes	17	63.0	7	50.0	31	62.0	15	40.5
	Metastasis	7	26.0	5	35.7	19	38.0	17	45.9
Spleen	Toxic changes	10	37.0	6	42.8	15	30.0	8	21.6
	Metastasis	4	15.0	4	28.5	5	10.0	5	13.5
	Toxic nephrosis	14	51.0	7	50.0	22	44.0	12	32.4
Kidneys	Pyonephrosis	3	11.0	4	28.5	5	10.0	5	13.5
	Metastasis	6	22.0	2	14.2	9	18.0	4	10.8
	Metastatic extension	3	11.0	1	7.1	6	12.0	2	5.4
Bladder	Pancreatitis	2	7.4	0	—	5	10.0	0	—
	Metastasis	5	18.5	1	7.1	8	16.0	3	8.1
	Toxic changes	5	18.5	—	—	7	14.0	—	—
Adrenals	Metastasis	6	22.0	4	28.5	12	24.0	10	27.0
	Metastasis	5	18.5	4	28.5	6	12.0	5	13.5
	Metastasis	2	7.4	1	7.1	7	14.0	7	18.9
G. I. Tract	Metastasis	2	7.4	1	7.1	7	14.0	5	13.5
	Edema	2	7.4	1	7.1	7	14.0	5	13.5
	Metastasis	10	37.0	3	21.4	16	32.0	—	—
Uterus	Metastasis	3	11.0	0	—	7	14.0	3	8.1
	Metastasis	7	26.0	3	21.4	19	38.0	7	18.9
	Metastasis	2	7.4	0	—	4	8.0	0	—
Ovary	Metastasis	—	—	—	—	—	—	—	—
	Metastasis	—	—	—	—	—	—	—	—
	Metastasis	—	—	—	—	—	—	—	—
Brain	Metastasis	—	—	—	—	—	—	—	—
	Metastasis	—	—	—	—	—	—	—	—
	Metastasis	—	—	—	—	—	—	—	—
Skelet.	Metastasis	—	—	—	—	—	—	—	—
	Metastasis	—	—	—	—	—	—	—	—
	Metastasis	—	—	—	—	—	—	—	—
Thyroid	Metastasis	—	—	—	—	—	—	—	—
	Metastasis	—	—	—	—	—	—	—	—
	Metastasis	—	—	—	—	—	—	—	—

When we turn to a consideration of both entire series, including the patients with cancer of the breast, as is shown in Table VI(B), we find the total incidence of bronchopneumonia is practically the same; 60 per cent in the refrigerated group and 53 per cent in the nonrefrigerated group. It is interesting to try to break down these figures in the refrigerated group in respect both to the presence of metastases and to the amount of generalized refrigeration the patients received.

What conclusions, if any, can be drawn from these figures in respect to the relationship of refrigeration and pneumonia? We have seen from the control patients that there is a normal expectancy of at least 50 to 60 per

cent of pneumonia in terminal malignancy, and that there is no appreciable difference in incidence between the persons with and without pulmonary metastases.

In the experimental series of 50 cases there occurred a total of 32 cases of bronchopneumonia. However, we must exclude those cases which occurred in patients who received only local refrigeration, i.e., 9 cases, as these cases could more properly be included in the control group. It is interesting to note that of the 15 patients treated only locally the incidence of pneumonia remains at the 60 per cent average level.

Of the 23 cases of pneumonia which occurred in patients who underwent "hibernation," 13 (56 per cent), or only slightly over half showed the presence of metastases. If we exclude the 7 patients with breast cancer who had pneumonia, we find that only 6 of the remaining cases (or 37 per cent) had metastases. In comparing these several figures analyzed variously, one can only come to the conclusion that the presence of pulmonary metastases does not significantly change the incidence of bronchopneumonia among patients undergoing generalized refrigeration. Likewise (and this is the point which above all we have been interested in determining), if these statistics can be accepted as of real significance, then they all point inevitably to the conclusion that generalized reduction of body temperature ("hibernation") does not change the incidence of terminal pneumonia in patients suffering from advanced malignant disease.

Liver, Spleen, and Kidneys.—In grouping these abdominal viscera together for consideration, we are prompted by the fact that such changes as have been noted have all been strictly comparable. There appears to be a slightly higher incidence of the so-called "toxic" degenerative reversible type of reaction observed in the refrigerated group than in the nonrefrigerated group. In other words, the mild, nonspecific granular changes seen so commonly in the liver and kidneys have been noted about 10 to 15 per cent more frequently in patients who have undergone "hibernation" than in those who have not. However, one must take into consideration the pneumonic incidence in relation to these changes, the amount of absorption from degenerating tumor tissue, the presence of metastases, and so many other variable factors that it seems only reasonable to assume that such minor changes as have been noted are within normal limits of variability. Certainly they do not appear to be sufficiently marked to be of any clinical importance.

Pancreas.—In the pancreas, on the other hand, it is our impression that we have pathologic changes among the refrigerated group which do not have their counterpart in the nonrefrigerated group. We might add that subsequent cases have given additional support to this contribution. While the actual incidence of pancreatic degenerative and inflammatory changes is only 10 per cent, it seems significant that *no* such changes have been found in the control group. These changes range from grossly apparent foci of fat necrosis or hemorrhage or both, to similar lesions of much less magnitude, demonstrable only microscopically. In line with Rich and Duff's⁷ conclusions, and more recently those of Smyth,⁸ these instances of so-called pancreatitis seem to

offer further substantiation to the theory of a vascular or circulatory origin in the development of the degenerative process. We feel that the slowed rate of blood flow may well be instrumental in producing tissue anoxia, followed by the characteristic pathology of the parenchyma of the organ. It most certainly offers a new approach to the experimental study of the condition.

As has already been commented upon, the following of these patients during "hibernation" by daily blood amylase determinations, as suggested by our biochemist, Dr. R. H. Hamilton, is a prophylactic measure which we have carried out routinely in subsequent studies. We are sure that at least one incipient severe instance of pancreatitis was prevented by this means, the patient being taken out of refrigeration with the sudden rise of the blood amylase to 1,100 units and the symptoms subsequently subsiding with the return of the amylase to normal levels.

Adrenal Glands.—No comparable control figures are available in respect to the adrenal glands. In going over the gross autopsy findings seldom was any comment found about any pathologic changes in these organs. Unfortunately, as a result microscopic sections were available in only a few instances. In view of the tremendous importance which Selye⁹ places upon the adrenal glands in the defense mechanism of the body, it is to be regretted that more detailed studies are impossible. Medullary depletion and cortical hyperplasia, as described by Selye, have certainly been observed in a number of the refrigerated cases, but irregularly. In any subsequent study more careful attention should and will be paid to these organs.

Brain.—Particular attention has been focused clinically upon the central nervous system of these patients during hibernation. The complete failure to recall anything over the entire period, the dulling of the sensorium, the slowing-up of speech and understanding, the complete mental recovery with relief of pain and headache, and not infrequently the almost euphoric state which has been noted, have all stimulated the keenest interest in neuropathologic studies.

Of the 50 patients coming to autopsy, permission was obtained to examine the brains of 44. Metastasis was found in only three cases; hence this factor does not enter prominently into the picture. Eleven cases should perhaps be excluded as the patients had received only local cold therapy. This still leaves 33 patients to be considered: 11 of these received less than 50 hours of general refrigeration, 17 received from 50 to 150 hours, and 5 had from 150 to 230 hours of "hibernation."

Outstanding as the only grossly demonstrable pathologic finding in the entire series was the presence of edema, which it must be admitted is often a very difficult thing to estimate. However, edema was recorded in 12 patients who had been refrigerated, and in an additional 4 who had received only local cold therapy. There is an interesting similarity in the percentage figures of the incidence of edema in (1) the group as a whole as shown in Table VI(B), i.e., 32 per cent; (2) 12 of the 35 persons refrigerated, i.e., 34 per cent; and (3) 4 of the 15 persons who were not refrigerated, i.e., 27 per cent.

From these figures it is apparent that generalized refrigeration in itself does not seem to be a significant factor in producing cerebral edema. The duration of the refrigeration period likewise does not appear to be significant in respect to either the incidence or the severity of the edema. In fact, the greater proportion of the cases were found in the less than 50 hour group.

Microscopic study has failed to reveal any other pathology. No changes affecting motor and sensory neurones or glial cells have been demonstrated. Nothing in the nature of an inflammatory encephalitic reaction has been noted. Indeed, both from clinical observation and from analysis of this pathologic material, we have been unable to date to demonstrate any harmful effects of refrigeration upon the central nervous system.

Metastases.—A perusal of the several tables reveals at a glance how far advanced the cases of malignancy in this series have been, for the percentage involvement, with the exception of the lungs, is considerably higher in each organ system than the figures given by most authors. Such a study further confirms the rather characteristic distribution of metastases in the various forms of cancer which have been encountered, but adds little else, in this respect, to our previous knowledge. From the standpoint of the effect of refrigeration upon these metastatic foci, we are scarcely warranted in hazarding an opinion, based on the data available in this material. Since most of the patients died, the duration of life was so seldom more than a few weeks that one would scarcely expect to find any convincing evidence of regression. Furthermore, it is impossible to have a truly representative base line—biopsied tissues—from the metastatic areas. Thus, changes which are demonstrable at autopsy either grossly or microscopically are not necessarily indicative of regression, the result of refrigeration. Only time and a sufficient number of cases can establish factual evidence in this regard.

It is true that regressive changes are likely to be observed in the metastatic tissues from the refrigerated group. It is equally true that similar changes may be observed in any malignant tumor. Undoubtedly, it is almost impossible to exclude bias entirely in a matter where one's interest and enthusiasm are so closely bound up. Nonetheless, it is our impression that in the majority of those patients subjected to more than 120 hours of "hibernation," the regressive cell changes seem to be more marked, and to occur with greater regularity in persons subjected to refrigeration than in those who were not. In no instance, however, has complete necrosis of any metastatic area been observed.

X-RAY STUDIES

The only possible way to evaluate accurately the gross regression of metastatic tumor is by means of serial x-ray studies where the size of the lesion can actually be measured at successive intervals. Through the splendid cooperation of the members of the Department of Radiology, especially Dr. W. E. Chamberlain, Dr. B. R. Young, and Dr. R. K. Arbuckle, such studies were undertaken whenever possible as a routine control yardstick for the

clinical and pathologic findings. Initial roentgenograms were obtained in 33 of the experimental group. In 14 of these no subsequent studies were obtained, in most cases because of the death of the patient before such repeat pictures were indicated. Of the 19 remaining patients, 9 must be eliminated because their treatment was by local cold application only. Of the remaining 10, three had less than 50 hours of refrigeration, and four others had from 50 to 150 hours; only three received over 150 hours of generalized refrigeration. No change was noted by x-ray in the group having less than 50 hours of refrigeration, nor in three who were in the second group (the 50 to 150 hour series); no apparent change was observed in one of the three (a case of breast cancer [E. B.] with widespread skeletal metastasis) who had as much as 230 hours of hibernation.

The other three cases perhaps merit individual comment. Case 15 (E. G.) was a patient with advanced breast cancer who received local treatment for several months, and then, in the terminal stages of her disease, was the first patient ever to undergo "hibernation." All that can be said is that during the last three months of her life there was no increase in the size of any of the skeletal metastases which in general had been progressive in character over the preceding six-month period.

Case 47 (A. W.) was a patient with carcinoma of the thyroid with widespread generalized and skeletal metastases, who was maintained under observation for about ten months. Most of his treatment was of the local type, but he did receive about 120 hours of generalized refrigeration. Following a three-month treatment period, the x-ray examination revealed definite improvement of many of the lesions, and the discovery of no new areas. Five months later improvement was still noted in the lesions in the cervical spine, the ribs and clavicle, but there was evidence of increased destruction in the pelvic bones.

Case 96 (L. Q.) was a patient who had the fulminating type of acute lymphatic leucemia that occurs in a child. He received no x-ray treatment other than that unavoidably required for repeated pictures. Over a two-month period he had several brief periods of refrigeration, following each of which there was a temporary measurable decrease in the mediastinal area of involvement, as well as of the spleen, accompanied by a profound drop in the white blood cell count. These regressive changes, however, were transitory and incomplete. Positive tissue cultures were obtained from cervical nodes at autopsy in spite of what grossly appeared to be complete liquefaction necrosis of the tissues.

Obviously, these data are totally inadequate with which to draw any conclusions in respect to the effects of generalized refrigeration upon metastatic tumor tissue. From such data as we do have it becomes apparent that no significant changes are likely to occur in much less than 240 hours of such low temperature application. Whether complete regression of such metastatic foci would ever result is extremely unlikely, but it does not seem unreasonable to feel that generalized hypothermy might well be an important adjunct to other forms of therapy. Its further exploration is urged.

SUMMARY

1. The histopathologic findings of 50 patients with malignant disease subjected to local, generalized, or combined refrigeration are compared to those of 37 closely analogous terminal cases of malignancy given only the usual supportive treatment during the terminal stages.

2. Critical analysis of the heart, lung, liver, spleen, and kidney findings reveals no very significant differences in the two groups.

3. The occurrence of acute pancreatic changes, either gross or microscopic, in about 10 per cent of the persons given refrigeration is particularly discussed from the standpoints of etiology and prevention.

4. The effect of réfrigération upon metastatic disease, as demonstrated by serial x-ray as well as autopsy studies, is discussed. It is suggested that at least 240 hours of such generalized refrigeration is apparently needed to induce any significant regression of such metastases, and that such regressions are of irregular occurrence only.

5. The further exploration of the use of reduced temperature as an adjunct to other forms of treatment of cancer is urged.

Acknowledgment should be made to a very considerable number of the hospital staff for their respective contributions in the conduct of these studies, but especially to Dr. Temple Fay and Dr. Augustus McCravey for their clinical observations; and to the members of the Pathology Department, Dr. E. E. Aegerter, Dr. H. C. Lennon, Dr. A. R. Peale, and Dr. L. L. Lawry, for their help in the performance of the autopsies. Dr. C. L. Brown as internist, Dr. W. E. Burnett as surgeon, Dr. H. Roesler as cardiologist, and Dr. G. C. Henny as physicist, likewise are particularly deserving of our gratitude.

REFERENCES

1. Fay, T., and Henny, G. C.: Correlation of Body Segmental Temperature and Its Relation to the Location of Carcinomatous Metastases, *Surg. Gynec. & Obst.* 66: 512, 1938.
- Smith, L. W., and Fay, T.: Temperature Factors in Cancer and Embryonal Cell Growth, *J. A. M. A.* 113: 633, 1939.
2. Warren, Stafford L.: Personal communication.
3. Oppenheimer, N. J., and McCravey, A.: Circulation Time in Man at Low Temperatures, *J. Physiol.* 129: 434, 1940.
4. Kossman, C.: General Cryotherapy, A Symposium: Cardiovascular Aspects, *Bull. New York Acad. Med.* 16: 317, 1940.
5. Smith, L. W.: Refrigeration in Cancer—Pathological Observations, *New York State J. Med.* 40: 1355, 1940.
6. Fay, T.: Observations in Prolonged Human Refrigeration, *New York State J. Med.* 40: 1551, 1940.
7. Rich, A. R., and Duff, G. L.: Experimental and Pathological Studies on the Pathogenesis of Acute Hemorrhagic Pancreatitis, *Bull. Johns Hopkins Hosp.* 58: 212, 1936.
8. Smyth, C. J.: Etiology of Acute Pancreatitis, *Arch. Path.* 30: 651, 1940.
9. Selye, H., Dosne, Christianne, and Bassett, Luch: On the Therapeutic Value of Adrenal Cortical Hormones in Traumatic Shock and Allied Conditions, *Canad. M. A. J.* 43: 1, 1940.

CLINICAL AND EXPERIMENTAL

THE DEVELOPMENT OF EOSINOPHILIA FOLLOWING LIVER THERAPY*

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INTRODUCTION

SINCE the institution of liver in the treatment of pernicious anemia, it has been a well-known fact that eosinophilia sometimes develops following the administration of large amounts of raw or cooked whole liver. That eosinophilia may follow the oral administration of liver extract has remained a debated issue. A review of the literature has revealed no reference to the development of eosinophilia following the administration of parenteral liver extract.

In 1927 Watkins and Berglund⁴ reported the development of a progressively rising eosinophilia in a patient with pernicious anemia. This patient had been placed on a raw liver diet. Up to the eighteenth day of observation the percentage of eosinophiles had remained relatively constant, but from that date on, frequent differential counts through the fiftieth day revealed a progressive eosinophilia to about 48 per cent. Whitby⁶ in 1928 reported the development of eosinophilia in four cases of pernicious anemia following the institution of liver therapy. Two patients had received raw liver only. One patient had received a combination of raw and cooked liver, and one had received cooked liver only. Eight to eleven weeks after institution of therapy a rise in eosinophiles to 6 to 8 per cent was noted. In one patient receiving raw liver alone a maximum of 26 per cent eosinophiles was attained. Smith and Whitby³ reported the occurrence of eosinophilia in patients convalescing from various medical and surgical conditions after they had received a diet of cooked liver over a period of from six to forty days. A maximum rise to 61 per cent was noted in one instance, but this was in a patient with Hodgkin's disease. Meulengracht and Holm¹ concluded that eosinophilia in the liver treatment of pernicious anemia appeared in a marked and persistent form when treatment was carried out with large doses of raw calves' liver and that this eosinophilia persisted as long as raw liver was given. The increase in eosinophiles in these cases ranged from 20 to 40 per cent, and in one case was as high as 74 per cent. Control patients suffering from various other diseases responded in a like manner. This eosinophilia was usually absent when fried liver or liver extract was employed. Whitby and Britton⁵ hold to the view that eosinophilia occurs only with the administration of raw liver and not with liver extract. Murphy²

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states that in patients ingesting either whole liver or liver extract there may occur an increase in the number of eosinophiles, in some cases to rather high levels. He reports one patient who received whole liver and in whom there developed an eosinophilia of 50 per cent of a total of 18,000 leucocytes. When this patient was given liver extract by mouth, there was some decrease, but not until the extract was given intramuscularly were normal values established.

REVIEW OF CASES OF PERNICIOUS ANEMIA SEEN IN THE STATE OF WISCONSIN GENERAL HOSPITAL

Since a review of the literature revealed few references to the development of eosinophilia following whole liver ingestion, no references to eosinophilia following parenteral liver therapy, and conflicting views regarding the development of eosinophilia following oral liver extract, it was felt that a study of the cases of pernicious anemia seen in the State of Wisconsin General Hospital from September, 1925, through September, 1938, might be instructive. This review was undertaken for the purpose of determining what form or forms of liver therapy produced eosinophilia, and to what extent.

TABLE I

ANALYSIS OF PERNICIOUS ANEMIA PATIENTS SEEN IN THE STATE OF WISCONSIN GENERAL HOSPITAL SEPTEMBER, 1925, THROUGH SEPTEMBER, 1938

TYPE OF THERAPY	TOTAL CASES	NUMBER WITH EOSINO-PHILIA	PER CENT OF TOTAL WITH EOSINO-PHILIA	PER CENT AVERAGE MAXIMUM EOSINO-PHILIA	REMARKS
No specific treatment	45	24	53.3	5.9	Eosinophilia of unknown cause
Whole blood transfusions	9	2	22.2	20.8	Individual maximum eosinophilia 34.6% and 7.0%
Whole blood (intramuscular injections—5 c.c. twice a week)	1	1	100	30.0	Maximum eosinophilia obtained after 2 months
Whole cooked liver	13	10	76.9	8.6	
Extralín (Lilly)	1	1	100	8.0	
Cofron (Abbott)	1	1	100	10.0	
Oral liver extract No. 428—Lilly	2	0	0	0	
Oral liver extract No. 29—Valentine	7	3	42.8	6.0	
Oral liver extract No. 343—Lilly	30	5	16.6	6.8	
Mixed liver therapy	91	31	34.1	10.4	Various forms of oral and parenteral liver, individual maximum eosinophilia 50.0%
Intramuscular liver extract—Lilly	79	36	45.5	11.3	Individual maximum eosinophilia 45.0%
Total cases	279				
Total number of cases receiving liver					224
Total number of cases with eosinophilia after liver therapy (excluding 6 cases with allergic background)					87 (38.8%)

For the purpose of this study, as well as for the experimental work to be reported in this article, a percentage of eosinophiles in excess of four was considered abnormal.

A total of 279 cases of pernicious anemia was studied. Of these, 224 received some form or forms of liver therapy, and of this latter group 87 showed an eosinophilia at some time. The results are summarized in Table I.

As frequent differential counts were not routine, it was impossible to determine: (1) the interval between institution of liver therapy and the beginning of eosinophilia, and (2) whether or not there occurred a gradual progressive eosinophilia throughout the period of liver treatment or merely a sporadic increase in eosinophiles.

EXPERIMENTAL STUDIES

An effort was made to study more closely the eosinophilic response to various forms of liver treatment in well or nearly well individuals, and to compare this response to that seen in patients with pernicious anemia who had received no liver therapy and from whom no allergic history could be obtained.

METHODS

In studying the four control cases, seven different "regimens" were employed. Two patients received 0.5 c.c. of Lilly's reticulogen intramuscularly every other day for a total of seven injections. Two patients received 3.0 c.c. of Lilly's liver extract intramuscularly every other day for seven injections. Three patients received one-half pound of cooked whole beef liver daily for a period of fourteen days.

The patients with pernicious anemia were treated according to the regular schedule for uncomplicated pernicious anemia. They received 3.0 c.c. of Lilly's extract intramuscularly every fifth day throughout the entire period of their hospitalization.

All patients were followed for at least one month. Total leucocyte counts were made in duplicate. The percentage of eosinophiles was determined each time by counting a total of 400 cells on three cover slip smears stained by Kingsley's method. In all persons with pernicious anemia and in some of the controls counts were made daily. In no instance were counts made less than three times each week, and they were always made at approximately the same time of day (2 P.M.).

In an effort to establish or rule out any correlation between skin reactions to liver extract and pathologic increases of eosinophiles following liver therapy, skin tests were done in most experiments before institution of treatment and again at the end of one month of therapy. For these skin tests 0.05 c.c. of Lilly's liver extract was injected intracutaneously. Experience revealed that the maximum reaction occurred after five minutes. It was arbitrarily decided that any reaction showing pseudopods should be taken as positive. The appearance of wheals per se was not considered representative of a positive reaction, since fourteen of fifteen normal tested individuals developed wheals.

RESULTS

An analysis of the control and anemic cases, with a brief résumé of each, follows:

A. Controls.—CASE 1. White male, 32 years old (95023). Diagnosis: delayed union of fractured tibia. The patient was seen in the hospital on five previous admissions when 16 total and differential white blood cell counts were made, and only once was an elevation of

eosinophile count above normal noted, and that to 5 per cent. The initial skin test revealed a wheal approximately 25 mm. in diameter without pseudopods. Lilly's liver extract regimen was then instituted after an initial blood count (see Chart 1). Fifteen days after the beginning of treatment an eosinophilia of 4.25 per cent was noted. A maximum response of 7.75 per cent was attained on the twenty-first day. The skin test at the end of the first month remained essentially unchanged with no pseudopods. After a rest period of ten days the patient received whole cooked liver irregularly for one week, followed by a period of two weeks during which he was given one-half pound of whole cooked liver daily. On the thirtieth day following the institution of the whole cooked liver regimen, a maximum eosinophilia of 7.25 per cent was attained. As shown in Chart 1, a progressive eosinophilia did not occur following either regimen, and with the intramuscular extract eosinophilia did not develop until after liver therapy had been stopped.

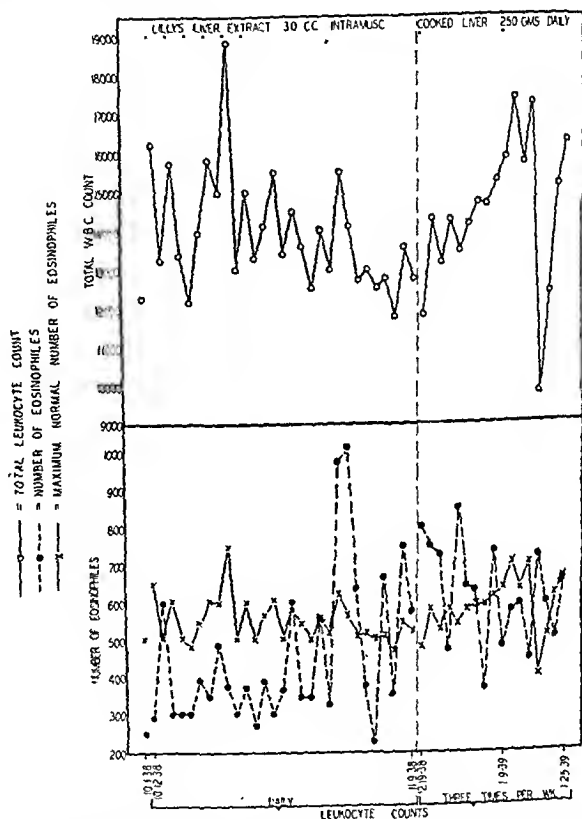


Chart 1.—The curves represent the leucocyte response in a normal individual during treatment with (1) liver extract intramuscularly, 3 c.c. every other day for seven doses; (2) whole cooked liver, one-half pound daily for a period of fourteen days.

CASE 2. White male, aged 22 years (9715). Diagnosis: healed lupus vulgaris (in for plastic repair). The initial skin test revealed no reaction whatsoever. The patient received Lilly's liver extract and later cooked liver, but at no time did the eosinophiles exceed normal limits.

CASE 3. White male, 29 years old (202906). Diagnosis: supracondylar fracture left femur, uncomplicated. The initial skin test revealed a wheal 10 mm. in diameter with no pseudopods. Observation for a period of one month following institution of reticulogen therapy revealed no eosinophilia.

CASE 4. White male, 49 years old (87237). Diagnosis: non-union fracture of left tibia. The initial skin test revealed a wheal 12 mm. in diameter without pseudopods. The patient received reticulogen and subsequently cooked liver. With each of these the eosinophile count reached a maximum of 5 per cent on two occasions. These increases were not considered to be significant.

Analysis of Control Cases.—Of the four control patients only one, Case 1, showed a definite eosinophilia and that was of approximately the same degree following cooked liver and intramuscular liver extract. Skin tests before and after the first regimen were considered to be negative. Skin tests on the remaining three controls were also negative and in none of these did an eosinophilia develop.

B. Pernicious Anemia.—CASE 1. White male, 38 years old (107597). The initial skin test revealed a wheal 17 by 17 by 2 mm. with no pseudopods. The patient did not develop eosinophilia during the month's observation when he received 3 c.c. of Lilly's liver extract intramuscularly every five days.

CASE 2. White male, aged 65 years (206698). The initial skin test revealed a wheal 15 by 16 by 2 mm. with two pseudopods about 2 mm. in length. Only one differential leucocyte count was obtained before institution of therapy. The patient developed a slight eosinophilia on the sixth day of parenteral liver extract therapy, but this was irregularly present thereafter with a maximum eosinophilia of 5.75 per cent on the thirteenth day. This level was not considered to be significant. A skin test at the conclusion of the experiment was not obtained.

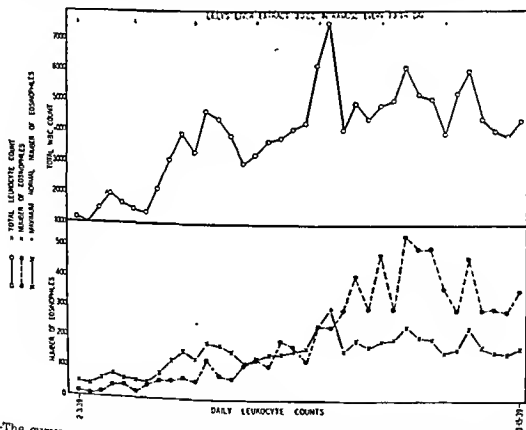


Chart 2.—The curves represent the leucocyte response in a patient with pernicious anemia, previously untreated, while receiving 3 c.c. of liver extract intramuscularly every fifth day.

CASE 3. White female, 50 years old (27360C). The initial skin test showed a wheal 20 by 15 by 2 mm. with three pseudopods, two of which were 10 mm. in length and 2 mm. in width. The third pseudopod was 2 mm. in length. Only one differential count was obtained before parenteral liver therapy was started. Chart 2 depicts the development of the progressive eosinophilia beginning on the eighteenth day and attaining a maximum of 9.75 per cent on the twenty-eighth day. Although liver therapy was continued, the subsequent level of eosinophilia through the thirty-ninth day, the last day of observation, varied between 6.75 and 8.25 per cent.

CASE 4. White female, 60 years old (207666). The initial skin test showed a wheal 14 by 18 mm. with no pseudopods. The patient exceeded the normal percentage of eosinophils only twice during the month's observation, when she developed a maximum eosinophilia of 5.5 per cent. The skin test at the end of the period of observation revealed a wheal 14 by 13 by 1 mm. with two pseudopods, 2 by 1 mm. and 17 by 2 mm. That a profound eosinophilia would have developed with further observation can only be surmised.

CASE 5. White male, 72 years old (105381). The initial skin test revealed a wheal 14 mm. in diameter with one pseudopod 5 mm. in length. The patient's blood showed a normal eosinophile count throughout the period of observation. A final skin test revealed a wheal 12 by 15 by 0.5 mm. with no pseudopods.

Analysis of Cases With Pernicious Anemia.—Of the five patients with pernicious anemia only one, Case 3, developed a definite eosinophilia. Cases 2 and 3 had positive initial skin tests. Case 1 had a negative initial skin test and developed no eosinophilia. Case 4 had a negative initial skin test and developed no significant eosinophilia but had a positive terminal skin test. Case 5 had a positive initial skin test, developed no eosinophilia, and had a negative terminal skin test.

DISCUSSION

A review of the records of patients with pernicious anemia seen in the State of Wisconsin General Hospital revealed the development of an average maximum eosinophilia varying from 6 to 11.25 per cent in 87 of the 224 persons who had received various forms of liver, and in whom other causes of eosinophilia were eliminated. However, 24, or 53.3 per cent, of the 45 patients who had received no specific treatment and in whom no allergic basis could be discovered showed an eosinophilia, although the average maximum rise was to 5.9 per cent only. No persons in this series received raw liver alone. Of the persons treated with whole cooked liver, 76.9 per cent developed eosinophilia. This incidence of cases developing eosinophilia was higher than that for any group receiving other types of liver therapy. The lowest frequency of eosinophilia coincident with liver treatment was that following oral extract (Lilly's No. 343), being only 16.6 per cent. The instances given in Table I, where only one or two cases received a specific type of treatment, are not considered. Importantly, this review showed that 45.5 per cent of the patients who received intramuscular liver extract developed eosinophilia with an average maximum rise to 11.25 per cent. The degree of eosinophilia in this group surpassed that of any other receiving liver therapy.

It follows, therefore, that eosinophilia may occur in patients with pernicious anemia who have received no specific treatment and in whom there is no obvious allergic or parasitic basis (stool examinations for intestinal parasites were done routinely on all cases of pernicious anemia); that eosinophilia may develop following cooked liver and orally and parenterally administered liver extracts, but apparently more frequently following the former. In no type of therapy, however, is this eosinophilia a regular occurrence.

Although definite deductions could not be made from this small experimental series, the results tend to show that eosinophilia may follow intramuscular liver extract therapy. Further, it is borne out, in agreement with the previously held view,^{1, 3} that eosinophilia following liver therapy is not a response peculiar to pernicious anemia. No correlation could be found to exist between skin tests with liver extract and the development of eosinophilia in the peripheral blood. This, of course, does not prove that the eosinophilia is not an allergic phenomenon but rather that skin tests with liver extract, as with many food extracts, may be unreliable. The experimental cases show that eosinophilia may develop after liver therapy has been stopped.

SUMMARY

1. Of the 279 patients with pernicious anemia seen in the State of Wisconsin General Hospital from September, 1925, through September, 1938, 224 received one or more forms of liver therapeutically. Allergic states and parasitic infestation had been excluded in each instance. Of this group of 224 cases, 87 developed eosinophilia at some time or other. However, eosinophilia of lesser degree was found in 24 of 45 cases in whom no evidence of allergy could be found and who had received no form of liver up to the time eosinophilia was discovered. Results of this review are analyzed in Table I.

2. Four control patients, who had no systemic disease and from whom no allergic history could be obtained, were observed for periods of one month following institution of liver therapy. Only one developed an eosinophilia.

3. Five previously untreated patients with pernicious anemia were similarly observed. One person developed definite eosinophilia while receiving parenteral liver extract.

4. An attempt to correlate a local dermal reaction to liver extract and the development of eosinophilia was unsuccessful.

CONCLUSIONS

The feeding of whole liver and the administration of oral and parenteral liver extracts may be followed by the development of eosinophilia in patients with pernicious anemia as revealed by the analysis of the records of such patients seen in the State of Wisconsin General Hospital.

Experimental studies revealed that: (1) Eosinophilia may follow the administration of whole liver and parenteral liver extract in presumably normal individuals; (2) eosinophilia may occur in patients with pernicious anemia following the parenteral administration of liver extract; (3) eosinophilia so induced may persist after cessation of liver therapy.

It has not been demonstrated that the eosinophilia, sometimes found following the administration of liver, is an allergic response nor has this possibility been excluded. Further investigation will be necessary to establish the cause for eosinophilia resulting from liver therapy.

REFERENCES

1. Meulengracht, E., and Holm, S.: Eosinophilia in Liver Diet, *Am. J. M. Sc.* 179: 199, 1930.
2. Murphy, W. P.: Anemia in Practice, Philadelphia and London, 1939, W. B. Saunders Co., p. 135.
3. Smith, H. S., and Whitby, L. E. H.: Blood Changes With Liver Diet in Young Convalescents, *Lancet* 2: 277, 1928.
4. Watkins, C. H., and Berglund, H.: Analysis of Morphological Blood Changes in Pernicious Anemia Following Administration of Liver, *Proc. Soc. Exper. Biol. & Med.* 25: 209, 1927.
5. Whitby, L. E. H., and Britton, C. J. C.: Disorders of the Blood, ed. 2, Philadelphia, 1937, P. Blakiston's Son & Co., p. 92.
6. Whitby, L. E. H.: White Blood Count Changes Resulting From Treatment of Pernicious Anemia With Liver, *Lancet* 1: 285, 1928.

PRECIPITIN REACTIONS IN RHEUMATOID ARTHRITIS

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SINCE the clinical course and pathologic findings of rheumatoid arthritis differ from osteo-arthritis, it is reasonable to presume that they also have separate etiologic factors. In the etiology of rheumatoid arthritis, the contributing factors are many, such as environment, heredity, avitaminosis, faulty metabolism, infection, and psychic trauma. Of all these factors, infection is believed to play a prominent role, and the *Streptococcus hemolyticus* is implicated in some way as suggested by the agglutination reaction.¹ The infection hypothesis is bolstered also by the fact that rheumatoid cases show an elevated sedimentation rate, a rise in serum globulin and a lowering of serum albumin, an elevated filament-nonfilament count, and a positive agglutination and precipitin reaction to *Streptococcus hemolyticus*.²

In this study we are presenting a series of cases of arthritis in which streptococcus precipitin reactions were done. Comparable agglutination reactions have been done by numerous investigators. Nicholls and Stainsby³ in 1931, using a "typical strain" of *Streptococcus hemolyticus*, obtained a positive agglutination in "practically all cases." In the following year Clawson and Weatherby,⁴ and Dawson, Olmstead, and Boots,⁵ found that 59 and 67 per cent, respectively, of rheumatoid types presented a positive agglutination. Wainwright⁶ in 1934 observed that the blood sera of 90 per cent of 87 cases of rheumatoid arthritis were positive. He used both living and heat-killed antigens. Nearly all these men used a "typical strain" of *Streptococcus hemolyticus*.

METHOD

Using the hydrochloric acid extract as antigen, Neil and Hartung⁷ in 1937 showed the precipitin reaction to be comparable to the agglutination reaction, in which live bacteria were used. We, therefore, used the hydrochloric acid extract as antigen because of the difficulty in keeping a growth of streptococcus alive. The method of obtaining the extract from a culture of *Streptococcus hemolyticus*, using the Lauecfield method, Porge modification, is as follows: a liter of plain broth having pH 7.6 is inoculated with 6 c.c. of fresh growing broth culture of *Streptococcus hemolyticus* and incubated at 37° C. for eighteen hours. The broth culture is then centrifuged and the precipitate is suspended in 0.85 per cent sodium chloride. Normal hydrochloric acid is added to give a resulting concentration of twentieth-normal. This acidified suspension (volume approximately 15 c.c.) is immersed in boiling water for fifteen minutes, then cooled to room temperature and centrifuged for thirty minutes. The resulting

supernatant is neutralized with sodium hydroxide, and the precipitate is discarded after centrifuging. The crystal-clear, slightly yellowish supernatant is the crude antigen and is ready for use.

The type-specific fraction designated as "M" may be obtained from this crude antigen by a series of extractions, using ethyl alcohol and sodium acetate. However, since Neil and Hartung⁷ in their study demonstrated that the crude antigen gave as many positive precipitin tests as the refined alcoholic extract containing the type-specific "M" fraction, and less than 0.1 per cent carbohydrate, only the crude antigen was used in this series. Three different antigens were used in the course of these precipitin reactions. The organism used in all three antigen preparations was a *Streptococcus hemolyticus*, beta type, freshly isolated from pathogenic sources.

TABLE I

PRECIPITIN REACTION IN PATIENTS WITH RHEUMATOID AND MIXED ARTHRITIS

CASE NO.	PATIENT	SEX	AGE	DURATION OF DISEASE	PRECIPITIN	TYPE	REMARKS
1	F. M.	F	68	15 years	000	Mixed	Arthritis practically burned out
2	Miss C. T.	F	45	4 years	X00	Rheumatoid	
3	Mrs. B.	F	49	16 years	XXX	Rheumatoid	
4	C. C.	F	56	9 years	000	Rheumatoid	
5	A. Boe	F	64	8 years	000	Mixed	Arthritis practically burned out
6	M. Bas.	F	23	9 months	000	Rheumatoid	
7	L. F.	F	56	9 months	XXO	Mixed	
8	C. F.	F	46	3½ years	XXX	Rheumatoid	
9	M. M.	F	43	3 years	XXX	Rheumatoid	
10	Miss P.	F	38	8 years	XXX	Rheumatoid	
11	Miss Tim	F	70	14 years	000	Rheumatoid	
12	Mrs. P.	F	64	18 years	X--	Rheumatoid	
13	Mrs. M. B.	F	47	2 years	000	Mixed	
14	M. R.	F	66	2 years	XXO	Mixed	
15	M. Bo.	F	52	6 months	000	Mixed	
16	A. Bas.	F	26	3 years	XXO	Rheumatoid	
17	Mrs. G. St.	F	53	3 years	XXX	Rheumatoid	
18	Mrs. J. Ha.	F	37	7 months	000	Rheumatoid	
19	T. L.	M	33	5 years	XXO	Rheumatoid	
20	S. W.	F	46	1 year	000	Rheumatoid	
21	Mr. V. D.	M	36	5 months	X00	Rheumatoid	
22	E. Y.	F	62	8 years	X00	Rheumatoid	
23	F. P.	F	44	24 years	X00	Rheumatoid	
24	L. T.	M	62	3 years	XXX	Rheumatoid	
25	W. R. R.	M	67	3 weeks	XX-	Rheumatoid	
26	R. N.	F	48	3 weeks	X00	Rheumatoid	
27	E. B.	F	60	25 years	XXX	Mixed	
28	J. MacP.	F	47	4 weeks	XXO	Mixed	
29	K. H.	F	64	9 years	XXO	Rheumatoid	
30	D. J.	M	35	2 months	X00	Rheumatoid	
31	M. F.	M	52	1½ years	X00	Rheumatoid	
32	E. Ois	F	28	4 years	000	Rheumatoid	

RESULTS

In 32 cases of the rheumatoid or mixed type there were 22, or 68.7 per cent, whose sera gave positive precipitin reactions. These results compare favorably with those of other workers, mentioned previously in this report. The 13 cases of osteo-arthritis, with but a few exceptions, gave negative results. The normal controls were also all negative.

TABLE II
PRECIPITIN REACTIONS IN PATIENTS WITH OSTEO-ARTHRITIS

CASE NO.	PATIENT	SEX	AGE	DURATION OF DISEASE	PRECIPITIN	REMARKS
1	Mrs. E. A.	F	56	2 years	000	Agglutination, negative 1-80
2	Mrs. C. D.	F	66	8 months	000	
3	A. C.	F	52	6 months	000	
4	L. H.	F	56	8 years	XXX	Also had varicose ulcer
5	Mrs. M. K.	F	53	2 months	X00	
6	E. K.	F	38	4 years	000	
7	J. Le.	M	62	2 months	000	
8	J. Li.	M	49	8 years	000	
9	Miss T.	F	73	10 years	000	
10	Mrs. P.	F	61	5 years	XXX	
11	L. C.	M	60	6 months	000	
12	S. H.	M	50	10 days	000	
13	F. P.	F	49	10 years	000	
14	A. Sig.	F	80	8 years	000	
15	M. A.	F	53	2 months	X00	

TABLE III
PRECIPITIN REACTIONS IN NORMAL CONTROLS

CASE NO.	PATIENT	SEX	AGE	PRECIPITIN
1	M. C.	F	23	000
2	D. H.	F	25	000
3	N. A.	F	24	000
4	J. G. B.	M	34	000
5	W. L.	M	32	000
6	J. Z.	M	23	000
7	S.	M	25	000
8	V. S.	F	24	000
9	L. S.	M	28	000
10	K. K.	M	25	000

Thus, it would seem that in the light of our present knowledge concerning arthritis, the *Streptococcus hemolyticus* is in some way implicated. Arthritis has been produced experimentally in rabbits by injecting small amounts (0.1 to 2 c.c.) of a broth culture of *Streptococcus hemolyticus* intravenously. The sedimentation rates, as well as the streptococcus agglutinins, were increased in these animals, whether they developed arthritis or not.⁸ These observations tend to make one suspect the streptococcus as an etiologic factor, except that the pathology produced in the joints clears up in a short time.

However, it is more likely that the agglutination test is nonspecific. The reaction is highly characteristic for atrophic arthritis, and Hench says "does not bar the conclusion that the agglutinins present in these sera are the result of rheumatoid disease."⁹ The Wassermann reaction is nonspecific in the sense that it is not a specific test for the presence of the spirochete. By the same token the agglutination and the precipitin reactions may be specific for atrophic arthritis, but without etiologic significance.

Other workers¹⁰ have shown that the agglutination titer may change from month to month on the same patient without any change in the sedimentation rate or clinical course of the disease. These men feel that the sedimentation rate is better as a diagnostic test than the agglutination reaction.

The ubiquitous streptococcus can be implicated; this is about all that can be said. Most authors agree with this postulate. More work to uncover the exact etiology of rheumatoid arthritis will have to be done before the exact role which the streptococcus plays can be determined. For the present, we may only say that the precipitins, as well as the agglutinins, in the sera of patients with rheumatoid arthritis are indirect evidence that infection plays a role in the etiology of this disease.

CONCLUSIONS

1. In a series of 32 cases of rheumatoid arthritis, the sera of 22 showed precipitins to the *Streptococcus hemolyticus* in high titer.
2. Four out of 15 cases of osteo-arthritis had a positive precipitin reaction, and none of the normal controls were positive.
3. The precipitin and agglutination reactions to the *Streptococcus hemolyticus* are indirect evidence that the streptococcus plays a part in rheumatoid arthritis.

REFERENCES

1. Boots, Ralph H.: The Essential Features in the Differential Diagnosis of Rheumatoid and Osteoarthritis, *J. LAB. & CLIN. MED.* 22: 18, 1939.
2. Hartung, E. F., Davis, J. Z., Steinbrocher, O., and Straub, M. E.: The Blood in Arthritis, *J. A. M. A.* 106: 1448, 1936.
3. Nicholls, E. E., and Stainsby, W. J.: Streptococcal Agglutinins in Chronic Infectious Arthritis, *J. Clin. Investigation* 10: 323, 1931.
4. Clawson, B. J., Wetherby, M., Hilbert, E., and Hilleboe, H. E.: Streptococcal Agglutination in Chronic Arthritis and Acute Rheumatic Fever, *Am. J. Med. Sc.* 184: 768, 1932.
5. Dawson, M. H., Olmstead, M., and Boots, R. H.: Agglutination Reactions in Rheumatoid Arthritis, *J. Immunol.* 23: 187, 1932.
6. Wainwright, Charles W.: Treatment of Chronic Atrophic (Rheumatoid) Arthritis With Streptococcus Vaccine on the Basis of Skin Sensitivity, Third Conference on Rheumatic Disease, *J. A. M. A.* 103: 1733, 1934.
7. Neil, Margaret Straub, and Hartung, E.: Precipitins for Streptococcus hemolyticus in Rheumatoid Arthritis Serums, *J. LAB. & CLIN. MED.* 22: 881, 1937.
8. Angevine, D. Murray, Cecil, Russell L., and Rothbard, Sidney: Immunologic and Bacteriologic Observations in Experimental Streptococcal Arthritis, *J. A. M. A.* 111: 2041, 1938.
9. Hench, P. S., Bauer, W., Dawson, H., Hall, F., Holbrook, W. P., and Key, J. A.: The Problem of Rheumatism and Arthritis—Review of American and English Literature for 1937, *Ann. Int. Med.* 12: 1005, 1939.
10. Short, C. L., Dienes, L., and Bauer, W.: Rheumatoid Arthritis—A Comparative Evaluation of the Commonly Employed Diagnostic Tests, *J. A. M. A.* 108: 2087, 1937.

LEUCOCYTE CHANGES IN ACUTE PERITONEAL IRRITATION*

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SINCE the introduction of the Schilling count¹ into clinical hematology, there has been much interest in the so-called "shift to the left." This is a term used to indicate an increased percentage of immature polymorphonuclear leucocytes in the differential count. The adult polymorphonuclear cells have two or more distinct lobes, connected by a very fine, threadlike filament. Any polymorphonuclear cell without this definite filament is not adult, even if there are two or more lobes. There are many different terms used to contrast these two types of cells, such as mature and immature; adult and young; segmented and nonsegmented; segmenters and band forms; filamented and nonfilamented; segmented and juvenile plus stab or staff cells. It is now customary to group together the juvenile and stab cells of Schilling's classification, and to call all polymorphonuclear cells either mature or immature.^{2, 3}

There is considerable difference of opinion concerning the normal percentage of immature polymorphonuclear leucocytes in the capillary blood. Some^{1, 4, 5} feel that normally there are only 3 to 6 per cent of these cells compared to the total number of white cells; and that anything above 5 per cent should be considered a shift to the left. Most of the more recent observers, however, follow the teaching of other authorities^{2, 6-8} and find a normal of 5 to 10 per cent, with the upper limit at 10 to 12 per cent. We consider 4 to 12 per cent normal; 13 to 20 per cent a slight shift; 21 to 39 per cent a moderate shift; and 40 per cent and over a marked shift.

The percentage of immature polymorphonuclear leucocytes has considerable diagnostic and prognostic value.^{1, 3, 9, 10} It is often assumed that a shift to the left indicates only the presence of infection. However, a shift may also be found in rupture of a viscus, thrombosis (coronary and elsewhere), hemorrhage, pregnancy, malignant neoplasms, acute ileus,¹¹ bone marrow irritation, such as that due to metastatic infiltration, and following various types of acute peritoneal irritation.

Since 1933, when the Schilling differential count was introduced as a routine in this hospital, we have been impressed with the constancy of the left shift, often extremely marked, in cases of acute peritoneal irritation. This observation has at times aided considerably in diagnosis. We, therefore, think it of interest to report a series of 132 differential counts in a group of 104 patients with various kinds of acute peritoneal irritation, proved by operation or autopsy.

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Several sources of error in our data must be recognized. The counts were made by 58 interns; hence the human error must be considered. In addition, there is a moderate statistical error inherent in counting only 100 to 200 cells.^{12, 13} As a result of these factors, the figures represent the usual conditions of medical practice in most hospitals.

"PRIMARY" PERITONITIS

The group of 9 cases of "primary" peritonitis is most illuminating in this connection. By primary peritonitis we mean "those cases which suddenly develop peritonitis unassociated with evidence of a pre-existing abdominal inflammation."¹⁴ Of the 9 counts (Table I), all except one (32 per cent) show a marked increase in immature polymorphonuclear leucocytes (44 to 74 per cent). The patient with only 5,750 white blood cells had the highest percentage of immature cells (74 per cent).

TABLE I
"PRIMARY" PERITONITIS

CASE	W.B.C.	POLYS. %	IMMAT. %	ONSET OF SYMPTOMS TO TIME OF COUNT
1	5,750	92	74	10 hours
2	18,900	64	32	24 hours
3	21,000	98	44	11 hours
4	23,150	89	50	18 hours
5	24,750	82	47	72 hours
6	24,800	95	58	4 hours
7	29,600	88	72	5 days
8	34,000	99	47	4 days
9	35,000	98	44	15 hours
Average		89.4	52.0	

PERFORATED PEPTIC ULCER

Table II shows the counts of 24 cases of perforated peptic ulcer. It will be noted that the shift is not so constant in this group of cases as in the preceding. On the whole, however, there is a definite trend for a rather marked shift. One example will suffice to show how rapidly the immature cells may be poured into the peripheral blood after a perforation of an abdominal viscus.

A 38-year-old man was admitted to the hospital for a partial pyloric obstruction from a cicatrized ulcer. There was no suggestion of a perforation; his temperature and pulse were normal; his differential count showed 86 per cent polymorphonuclear cells, of which only 8 were immature. Twenty-four hours after admission the patient complained of sudden onset of terrific epigastric pain, and the diagnosis of perforated peptic ulcer was made clinically. Fifteen minutes after the onset of pain one of us did a differential count and found 92 per cent polymorphonuclear cells, of which 24 were immature. One and a half hours after the perforation, just before operation, the count was repeated by the same observer, and there were now 97 per cent polymorphonuclear cells, of which 39 were immature. Operation confirmed the diagnosis.

PERFORATION OR GANGRENE OF SMALL INTESTINE

We have recorded in Table III the results of 17 counts done on 6 patients with perforation or gangrene of the small intestine. These persons, including those with gangrene in whom a definite perforation could not be found, all had purulent peritoneal fluid containing organisms. The large number of counts

TABLE II
PERFORATED PEPTIC ULCER

CASE	W.B.C.	POLYS. %	IMMAT. %	ONSET OF SYMPTOMS TO TIME OF COUNT
10	9,000	67	18	3 hours
11	9,350	75	31	1 hour
12	9,960	91	46	8 hours
13	12,100	95	22	10 hours
14	13,000	90	30	1 hour
15	13,650	90	22	18 hours
16	13,750	98	39	3 hours
17	14,500	85	46	24 hours
18	14,750	90	9	6 hours
19	15,000	78	28	4 hours
20	15,050	88	47	2 hours
21	15,200	90	20	10 hours
22	15,650	88	47	5 hours
23	15,800	84	26	17 hours
24	15,800	80	32	4 hours
25	16,500	94	36	6 days
26	16,550	94	81	16 hours
27	18,600	66	13	3 hours
28	18,700	87	18	6 hours
29	19,150	98	18	5 hours
30	21,800	92	36	4 hours
31	22,500	78	18	10 hours
32	26,400	88	32	7 hours
33	Not done	92	24	4 hour
33	Not done	97	30	14 hours
Average		86.5	31.1	

TABLE III
PERFORATION OR GANGRENE OF SMALL INTESTINE

CASE	W.B.C.	POLYS. %	IMMAT. %	ONSET OF SYMPTOMS TO TIME OF COUNT
34	3,650	64	63	?
34	4,700	65	48	6 hours
35	5,600	90	40	34 days
36	7,000	79	40	4 days
37	7,050	59	37	5 days
35	7,200	74	27	54 hours
38	7,300	79	69	7 days
36	10,000	48	30	5 days
35	13,200	84	29	30 hours
35	15,000	90	10	6 hours
39	17,200	84	24	15 hours
39	17,800	86	18	4 hours
35	18,000	96	24	18 hours
37	19,500	92	24	12 hours
39	24,000	90	28	10 hours
37	28,500	80	22	24 hours
36	Not done	61	44	6 days
Average		77.7	34.0	

per person indicates the difficulty of arriving at a diagnosis in this type of case, and the consequent delay in operating. It is important to note that in the cases with a low total white blood cell count the shift to the left is usually most marked. The practical significance of this observation is evident, since it is in those cases with a low total white blood cell count that the surgeon tends to delay unnecessarily. An example will illustrate this point.

A 39-year-old German bartender was admitted to the hospital with a history of four days of dull, intermittent midabdominal pain, anorexia, nausea and frequent vomiting. For the past ten years he had had a right inguinal hernia, which had never given him any trouble.

On examination he was found to have marked congestion of the tonsils and pharynx, a few small axillary and epitrochlear nodes, and a right indirect inguinal hernia with a relaxed external ring, admitting one finger. His temperature was 101.2° F. and his pulse was 100 on admission, but both came down to normal within twelve hours and remained normal. The initial blood count showed 7,000 white blood cells; polymorphonuclear cells 79 per cent, of which 40 were immature; lymphocytes 21 per cent. The next day the total count was 10,000; polymorphonuclear cells 48 per cent, of which 30 were immature; lymphocytes 42 per cent; monocytes 6 per cent; eosinophiles 4 per cent. The following day the total white blood cell count was not determined, but the differential count showed polymorphonuclear cells 57 per cent, of which 40 were immature; myelocytes 4 per cent; lymphocytes 24 per cent; monocytes 11 per cent; eosinophiles 4 per cent. During the first four days in the hospital he had no symptoms, but on the fifth day he developed pain in the right inguinal region. The external ring was found filled with intestines. Shortly thereafter the patient reduced the hernia himself. Operation was nevertheless advised, but was not considered urgent. The patient insisted on going home, for he felt well and wished to return to work. The next afternoon he returned in shock, after having vomited all night. He died two hours after readmission. Autopsy revealed two areas of gangrene, 6 cm. apart, in the distal ileum, one recent and the other old.

PERFORATED APPENDIX

The cases of perforated appendix, either without abscess or with an abscess of less than five days' duration, are listed in Table IV. In this group also there is a tendency for those cases with a low total white blood cell count to have a more marked shift than those with a higher count.

To show how rapidly and dramatically the blood picture can change, we wish to mention the case of a 2-year-old female child who was admitted to the hospital with symptoms and signs suggesting an acute abdominal inflammation. The initial count, made forty-eight hours after the onset of symptoms, was as follows: white blood cells 7,800; polymorphonuclear cells 62 per cent, of which but 22 were immature; lymphocytes 38 per cent. This relatively normal count was in part responsible for a short delay in asking the surgeons to see the child. Five hours later the count was white blood cells 4,000; polymorphonuclear cells 80 per cent, of which 70 were immature; lymphocytes 20 per cent. Operation was performed immediately after this count, and a perforated appendix was found.

PERFORATION OF SIGMOID DIVERTICULI

In Table V we have recorded the results of 6 counts in 4 cases of perforation of sigmoid diverticuli. All the counts, with one exception, show a marked shift to the left. Here again the low total counts were accompanied by a high immature count, even when the polymorphonuclear cells were not markedly increased. The one case in which no significant shift was recorded had a total white blood cell count of 33,800 and a polymorphonuclear cell count of 93 per cent.

PERFORATED GALL BLADDER

It is well recognized by clinicians that the severity of most cases of acute cholecystitis is extremely difficult to evaluate, and that an empyema or perforation of the gall bladder may be present with very slight symptoms and signs or changes in the blood picture.¹⁵⁻¹⁷ In our small series of 6 proved cases of perforated gall bladder only 1 out of a total of 9 counts showed a greater percentage of immature cells than 32 (Table VI). It is unfortunate, but apparently true, that the degree of shift cannot be counted on as an aid in diagnosing the severity of a cholecystitis.

TABLE IV
PERFORATED APPENDIX

CASE	W.B.C.	POLYS. %	IMMAT. %	ONSET OF SYMPTOMS TO TIME OF COUNT
40	4,000	80	70	53 hours
41	5,400	65	34	72 hours
42	6,750	86	41	60 hours
43	7,150	94	74	48 hours
40	7,800	62	22	48 hours
44	9,100	86	40	72 hours
45	10,400	72	11	54 hours
46	10,600	59	25	4 days
47	11,500	90	52	5 days
48	11,850	91	29	5 days
49	13,200	86	35	24 hours
50	13,400	95	10	72 hours
51	13,700	85	33	36 hours
52	14,500	85	22	25 hours
53	14,700	85	48	48 hours
54	15,000	88	30	72 hours
55	16,200	95	40	48 hours
56	16,800	80	20	4 days
57	17,200	86	16	48 hours
58	17,700	93	10	4 days
59	18,400	90	32	72 hours
60	18,600	96	48	36 hours
61	18,800	88	26	24 hours
62	19,200	88	25	36 hours
63	19,900	76	15	48 hours
64	20,000	91	26	25 hours
65	20,450	89	28	4 days
66	21,400	90	25	4 days
67	24,200	90	9	14 hours
68	24,500	96	20	48 hours
69	24,800	90	29	6 hours
70	26,800	83	45	4 hours
71	27,100	92	68	28 hours
72	27,500	96	40	21 hours
73	29,000	85	28	?
74	30,700	88	19	23 hours
75	30,700	93	32	50 hours
Average		86.0	31.6	

TABLE V
PERFORATED SIGMOID DIVERTICULI

CASE	W.B.C.	POLYS. %	IMMAT. %	ONSET OF SYMPTOMS TO TIME OF COUNT
76	7,900	84	42	18 hours
77	9,090	76	50	52 hours
77	9,500	72	42	18 hours
77	13,950	86	57	40 hours
78	24,150	92	74	14 days
79	33,800	93	16	72 hours
Average		83.8	46.8	

HEMORRHAGIC PANCREATITIS

The cases of acute hemorrhagic pancreatitis in our series (Table VI) are too few to be discussed in detail. There is a tendency for an extremely high polymorphonuclear cell percentage and for a marked shift to the left, although the latter is not consistently noted. In typical instances of this disease, the aid which may be had from the blood count is unnecessary, as the clinical picture of

a surgical abdominal emergency is so striking. If one must attempt to distinguish between hemorrhagic pancreatitis and coronary occlusion, as occasionally happens, it would appear that a very high polymorphonuclear count and a marked shift, especially if they occur within a few hours after the onset of symptoms, favor the former diagnosis.

TABLE VI

CASE	W.B.C.	POLYS. %	IMMAT. %	ONSET OF SYMPTOMS TO TIME OF COUNT
<i>Perforated Gall Bladder</i>				
80	4,450	77	3	5 days
81	10,500	80	32	44 days
82	13,500	93	68	32 hours
83	15,800	86	12	2 hours
84	19,500	92	10	?
85	20,400	84	12	63 hours
86	22,500	96	30	45 hours
87	23,000	88	24	19 hours
88	38,000	96	24	21 hours
Average		88.0	23.9	
<i>Hemorrhagic Pancreatitis</i>				
86	13,600	89	42	16 hours
86	14,500	90	56	42 hours
87	15,900	90	46	?
88	21,000	98	29	72 hours
88	26,150	96	21	4 days
Average		92.6	38.8	

TABLE VII

CASE	W.B.C.	POLYS. %	IMMAT. %	ONSET OF SYMPTOMS TO TIME OF COUNT
<i>Ruptured Ectopic Pregnancy</i>				
89	5,650	76	38	42 hours
89	9,850	86	65	6 hours
89	11,300	76	17	20 hours
90	15,000	88	34	6 days
89	23,000	84	26	13 days
91	29,000	85	32	50 hours
Average		82.5	35.3	
<i>Ruptured Graafian Follicle and Corpus Luteum Cyst</i>				
92	7,500	78	6	15 hours
92	8,300	84	13	60 hours
93	8,400	80	15	25 hours
92	8,600	84	32	72 hours
94	9,800	87	25	1 hour
95	11,200	83	12	4 hours
96	13,400	71	18	4 days
97	14,100	84	52	9 hours
98	19,300	86	22	48 hours
Average		81.9	21.7	

RUPTURED ECTOPIC PREGNANCY, GRAAFIAN FOLLICLE, CORPUS LUTEUM CYST

A sudden and profuse hemorrhage into the peritoneal cavity may give rise to a marked shift to the left, similar to an infectious process; but usually if the hemorrhage is not large or sudden, the shift is not noteworthy. Three cases of ruptured ectopic gestation and seven cases of rupture of a Graafian follicle or

of a corpus luteum cyst are recorded in Table VII. The presence of a marked shift in a patient suspected of having had a rupture of an ectopic pregnancy is of considerable aid in deciding on an immediate operation. On the other hand, it is reassuring to note the low immature counts of the cases of rupture of a Graafian follicle or corpus luteum cyst, as these conditions do not require operation.

One patient who was known to be about two months pregnant had a white blood cell count of 9,850, with 86 per cent polymorphonuclear cells and 65 immature cells six hours after the onset of vague abdominal complaints. As the symptoms and signs were mild and as they rapidly subsided, she was allowed to go home. She returned thirteen days later, was immediately operated upon for a ruptured ectopic pregnancy, and died within a few days from uremia. If the extremely marked shift found on admission, and undoubtedly due to a sudden profuse intra-abdominal hemorrhage, had received due weight, this patient's life might well have been saved.

TABLE VIII

CASE	W.B.C.	POLYS. %	IMMAT. %	ONSET OF SYMPTOMS TO TIME OF COUNT	DIAGNOSIS
99	4,700	51	42	6 days	Volvulus
99	6,800	63	59	6½ days	Volvulus
100	8,600	95	83	6 hours	Site unknown
101	12,000	96	46	10 hours	Site unknown
102	14,300	87	41	12 hours	Perforated Fallopian tube
99	15,150	82	26	2 days	Volvulus
99	16,100	71	56	5 days	Volvulus
103	16,500	92	15	52 hours	Periappendicitis
104	20,600	91	42	72 hours	Periappendicitis
Average		80.9	44.2		

MISCELLANEOUS CASES OF ACUTE PERITONEAL IRRITATION

Various types of acute peritoneal irritation are gathered together in Table VIII, and it will be seen that the one thing these cases have in common is a sudden peritoneal insult. Most of them show the typical marked shift, which, when present with abdominal symptoms, is very important diagnostically. One case will well illustrate the value of understanding the significance of a high immature count in an acute abdominal condition.

A 61-year-old female had been on the medical ward for ten weeks with hypertensive cardiovascular renal disease. She had been decompensated on admission, but was doing very well when she was awakened one morning by rather severe generalized abdominal pain. She vomited several times, perspired profusely, and had cold extremities. Examination soon after the onset of symptoms revealed diffuse abdominal tenderness, especially in the left upper quadrant. Her temperature was 98.0° F.; her pulse was 70; and her respirations were 24 per minute. We were under the impression that we were dealing with an acute abdominal condition of some sort. A blood count, performed shortly after the examination or five hours after the onset of the pain, was as follows: white blood cells 8,600; polymorphonuclear cells 95 per cent, of which 83 were immature. Because of the extreme shift to the left, a diagnosis of acute peritonitis seemed almost certain. For this reason, an abdominal tap was immediately performed and 1 c.c. of turbid, straw-colored fluid with numerous flecks of fibrin was removed. A smear showed a variety of gram-positive and gram-negative bacilli and cocci, later substantiated by culture. The organisms obviously entered the abdominal cavity from the intestinal tract, probably as the result of a leak through the chronically distended gut or following a mesenteric thrombosis. Because of the patient's poor cardiovascular condition, operation was deemed inadvisable, and strangely enough, the patient made an uneventful recovery in a few days and subsequently went home.

DISCUSSION

We wish to emphasize that in the presence of suspected abdominal pathology, a high percentage (over 35 to 40 per cent) of immature polymorphonuclear leucocytes in the peripheral blood suggests acute peritoneal inflammation, almost always requiring immediate surgical intervention. In Table IX the immature cell percentages of all the counts are averaged according to the number of hours elapsing from the onset of symptoms to the time the count was done. This grouping brings out the fact that in this series the average immature count is almost identical in the group in which the counts were done shortly (less than ten hours) after the onset of symptoms and in the group in which many hours or even days elapsed. It is, therefore, apparent that the shift does not depend so much on infection as on sudden peritoneal shock or irritation. Even a very few hours after the perforation of a gastric ulcer, for example, there is usually noted a considerable shift. This is evidently not dependent on an infection, but is probably due to an inflammation of a chemical nature.

TABLE IX

ONSET OF SYMPTOMS TO TIME OF COUNT	AVERAGE PER CENT IMMATURE POLYS.
Under 10 hours	34.0
11-30 hours	33.3
Over 30 hours	34.3

TABLE X

TOTAL W.B.C.	AVERAGE PER CENT IMMATURE POLYS.
Under 10,000	41.2
10,000-20,000	30.2
20,000-30,000	34.7
Over 30,000	30.3

While one should consider the blood count as a whole, our experience with this series of patients makes us feel strongly—as do many others^{1, 2, 3}—that the degree of shift is the most important aspect of the hematologic picture in acute abdominal conditions, with the exception of gall bladder pathology. It must be realized, however, that a slight or absent shift does not by any means rule out shock or severe intra-abdominal disease. It is of the utmost importance to recognize the significance of a high percentage of immature polymorphonuclear leucocytes in the presence of a low total white blood cell count. It will be seen from Table X that the greatest shift on the average was in those cases in which the total white blood cell count was “normal,” i.e., under 10,000. It is often in the patients with normal total white blood cell counts that delay in operation seems safe to the surgeon, whereas less attention to the total and more attention to the degree of shift would aid considerably in diagnosis and prognosis.

CONCLUSIONS

1. A marked shift to the left in the differential blood smear is frequently present in all types of acute peritoneal irritation, even without infection.
2. The absence of a marked shift does not eliminate the possibility of acute peritoneal irritation.

3. In the presence of a low total white blood cell count, a definite shift is of especial importance in diagnosis and prognosis.

4. Repeated differential smears, properly interpreted, aid materially in deciding when and whether to operate for a suspected surgical abdominal emergency.

We wish to express our sincere thanks and appreciation to Dr. Henry Henstell for considerable aid in the preparation of the manuscript. Also to Miss Victoria Braun and her staff of the record room for their cheerful cooperation.

REFERENCES

1. Schilling, V.: *The Blood Picture and Its Clinical Significance*, St. Louis, 1929, The C. V. Mosby Co.
2. Farley, D. L., St. Clair, H., and Reisinger, J. A.: *The Normal Filament and Nonfilament Polymorphonuclear Neutrophil Count: Its Practical Value as a Diagnostic Aid*, *Am. J. M. Sc.* 180: 336, 1930.
3. Haden, R. L.: *The Interpretation of Qualitative Changes in Neutrophilic Leukocytes*, *Cleveland Clin. Quart.* 5: 184, 1938.
4. Osgood, E. E.: *Normal Hematologic Standards*, *Arch. Int. Med.* 56: 849, 1935.
5. Crocker, W. J., and Valentine, E. H.: *Hemography in Diagnosis, Prognosis and Treatment Based on 6,000 Schilling Hemograms*, *J. LAB. & CLIN. MED.* 20: 172, 1934.
6. Cooke, W. E., and Ponder, E.: *The Polynuclear Count*, Philadelphia, 1927, J. B. Lippincott Co.
7. Reznikoff, P.: *Immature White Blood Cell Counts in Infectious Diseases*, *J. A. M. A.* 93: 963, 1929.
8. Needles, R. J.: *A Neutrophilic Graph*, *J. LAB. & CLIN. MED.* 17: 962, 1932.
9. Schilling, V.: *Clinical Interest in the Enumeration of the Staff Cells*, *Rev. belge sc. méd.* 10: 264, 1938.
10. Klöckner, G.: *The Blood Picture as an Aid in Diagnosis and Indication for Operation of Atypical and Typical Cases of Peritonitis*, *Med. Welt.* 9: 1622, 1935.
11. Demidova, P. N.: *Blood Picture in Acute Ileus*, *Vestnik khir.* 56: 356, 1938 (abstracted in *J. A. M. A.* 112: 1775, 1939).
12. Mainland, D., Du Bilier, B., and Stewart, C. B.: *The Accuracy of Differential Blood Counts*, *Canad. M. A. J.* 33: 667, 1935.
13. Goldner, F. M., and Mann, W. N.: *The Statistical Error of the Differential White Count*, *Guy's Hosp. Rep.* 88: 54, 1938.
14. Duncan, J. H.: *Primary Peritonitis: Pneumococcic and Streptococcic*, *Canad. M. A. J.* 24: 778, 1931.
15. Douglas, J.: *Acute Inflammation of the Gall-Bladder and Biliary Ducts*, *New York State J. Med.* 37: 1119, 1937.
16. Wesson, H. R., and Montgomery, T. R.: *Acute Cholecystitis: Report of Eighty-seven Cases*, *Proc. Staff Meet., Mayo Clin.* 12: 500, 1937.
17. Glenn, F.: *Acute Cholecystitis*, *Surg., Gynec. & Obst.* 69: 431, 1939.

THE EFFECT OF BELLADONNA ON THE APPETITE OF PATIENTS WITH OBESITY AND WITH OTHER DISEASES*

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IT WAS noted in a previous report¹ that relative inactivity was an important factor in the production of excess body weight in a majority of obese patients. These findings indicate that the appetite was not reduced in proportion to the activity. That there is a disturbance in control of appetite in such patients is supported by the additional fact that many patients state that hunger prevents them from adhering to a low calorie diet. Our interest in the control of hunger by medication was aroused several years ago when a patient stated repeatedly that she was able to follow a low calorie diet only so long as she took a medication which contained belladonna and bromide. This observation caused us to prescribe tincture of belladonna alone, or in combination with bromide or with phenobarbital, to other patients without their knowledge of the purpose.

TABLE I

DIAGNOSIS	TINCTURE BELLA- DONNA AND BROMIDES		TINCTURE BELLADONNA		TINCTURE BELLA- DONNA AND PHENOBARBITAL		TOTAL
	APPETITE		APPETITE		APPETITE		
	DECREASED	NOT AFFECTED	DECREASED	NOT AFFECTED	DECREASED	NOT AFFECTED	
Obesity	24	3	3		7	2	39
Diabetes and obesity			1		2		3
Diabetes			1		1		2
Chronic pyelo- nephritis			1				1
Total	24	3	6	0	10	2	45

It is to be noted from Table I that the appetite was diminished in 40 of 45 patients following the administration of such medication. These results, we believe, are higher than would be obtained in a larger series of cases. That the medication was an important factor in reduction of appetite is shown by the fact that ten patients who omitted the medication of their own accord resumed it because they noted that hunger returned. The duration of the effect has varied from as short as three weeks to as long as two years. The duration of the effect in most patients appears to continue indefinitely as long as the medication is continued. One patient with diabetes and moderate obesity was of particular interest. She had never been able to control the diabetes because

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hunger caused her to eat between meals. The administration of tincture of belladonna, 10 drops three times daily, before meals, eliminated this hunger and permitted her to control the diabetes.

Another patient presented an unusual case. She was forced to limit her activity because of chronic pyelonephritis and hypertension. She began to gain weight following this diminished activity. The administration of tincture of belladonna decreased her food intake sufficiently to stop the gain in body weight. Omission of the drug at a later date was followed by gain in body weight and a constant body weight again followed the administration of the drug. The prescribed diet was not altered during these periods of observations, and she voluntarily stated that her appetite was diminished during the periods of medication.

The cause of the increase in appetite in patients with obesity is not known. Harrington² and Newburgh³ concluded from their studies that many patients ingested food at frequent intervals because of nervousness or of habit. Our observations support this contention in certain patients, and it is for this reason that a mild sedative is of distinct aid in controlling the appetite in such patients. We have had only three patients who were given phenobarbital alone. The appetite was not altered in two, but it was definitely decreased in the third.

The fact that belladonna decreased the appetite in certain patients suggested that an alteration of motility of the stomach or small intestine may be a contributing cause. A series of investigations regarding this point have been in progress in this clinic, and the results will be given in another report. Lesses and Myerson⁴ have reported that benzedrine will also diminish the appetite in certain patients. They state that this drug not only relaxes the stomach and intestinal tract, but also alters the mood of the patient and increases his sense of well-being. Atropine may possibly have some slight psychic effect in the doses administered in this study, but its principal effect is upon the gastro-enteric tract. These observations indicate that the action upon the gastro-enteric tract was the major one in decreasing the appetite of our patients with obesity.

SUMMARY

The administration of belladonna alone or with a mild sedative decreased the appetite in 40 of 45 patients. The relative number of patients affected undoubtedly would be less in a larger series of cases. The causes for the decrease in appetite are discussed.

REFERENCES

1. Greene, James A.: Clinical Study of the Etiology of Obesity, *Ann. Int. Med.* 12: 1797, 1939.
2. Harrington, Mary M.: Appetite in Relation to Weight, *J. Am. Dietet. A.* 6: 101, 1930.
3. Newburgh, L. H.: The Cause of Obesity, *J. A. M. A.* 97: 1659, 1931.
4. Lesses, M. F., and Myerson, A.: Benzedrine Sulfate as an Aid in the Treatment of Obesity, *New Eng. J. Med.* 218: 119, 1938.

TOXIC EFFECTS OF SULFANILAMIDE, SULFAPYRIDINE, AND RODILONE IN ANOXIA*

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EFFECTS of sulfanilamide on airplane pilots have interested Mackie¹ and Rook² in England, and their communications have received recent comment in the *Journal of the American Medical Association*.³ Mackie reported that a pilot taking a full dose of sulfanilamide just prior to flying exhibited symptoms of anoxemia at an altitude of 13,000 feet. Since the partial pressure of oxygen at this altitude is 97 mm., this corresponds to only a moderate degree of anoxia. These authors state that sulfanilamide or its derivatives lowers the aviator's ceiling, or tolerance to anoxia, by about 5,000 feet. Rook² notes that similar observations have been made in America and Germany. Mackie¹ suggests that the cause of such reactions is an idiosyncrasy in which methemoglobin or sulfmethemoglobin is formed in sufficient amount to decrease significantly the oxygen-combining capacity of the blood.

Apart from the practical aspects of this purported effect of sulfanilamide derivatives, the problem deserves further investigation because cyanosis produced by sulfanilamide is not generally considered to be due entirely to inactivation of hemoglobin, and the effects of anoxia on actions and toxicity of sulfanilamide have not been thoroughly studied. Accordingly, the acute and cumulative toxic effects of three sulfanilamide-type drugs were proposed for study in mice exposed to various degrees of anoxia.

ACUTE EXPERIMENTS

From four to five hours after receiving oral doses of one of the three agents, or of the vehicle, 225 adult white mice, weighing from 18 to 22 Gm. each, were exposed to varying degrees of anoxia. All agents were suspended in 10 per cent aqueous solutions of acacia, and the concentrations adjusted so that all mice received 50 ml. per kilogram of solution. Sulfanilamide was given in a single dose of 20 Gm. per kilogram, and both sulfapyridine and rodilone were given in single doses of 10.0 Gm. per kilogram. Control mice were treated with 50 ml. per kilogram of 10 per cent acacia solution. Intragastric administrations were made with a blunted No. 18 needle, as recommended by Molitor and Robinson.⁴ No deaths occurred immediately after treatment, showing that neither too rapid dilation of the stomach nor aspiration of solution into the lungs occurred.

Doses chosen were in the lower portion of the toxic range of the three compounds, for 3 of 64 mice treated with sulfanilamide, 3 of 59 treated with sulfa-

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pyridine, and none of 53 treated with rodilone died during the four- to five-hour period prior to exposure to anoxia. None of 60 control mice which received aeacia alone died before exposure to anoxia. Doses having a definite but low toxicity were purposely chosen to magnify any effects which anoxia might have.

Anoxia was produced in a decompression chamber resembling that of Kolls and Loevenhart.⁵ This tank allows adequate ventilation, and even at very low pressures the air is changed rapidly enough to prevent accumulation of carbon dioxide. From ten to fifteen minutes were allowed for slowly decreasing the tank pressure, according to the final pressure attained.

Control mice, treated with aeacia alone, were always exposed to low pressures simultaneously with mice treated with the drugs.

RESULTS

Four groups of 10 mice treated with sulfanilamide were subjected to anoxia for one hour. One group was exposed to a partial pressure of oxygen of 100 mm., the second group to a partial pressure of oxygen of 80 mm., the third group to a partial pressure of oxygen of 64 mm., and the fourth group to a partial pressure of oxygen of 53 mm. No deaths occurred at these levels, which correspond to approximate altitudes of 12,000, 18,000, 24,000, and 28,000 feet, respectively. All of 20 treated mice survived exposure to a partial pressure of oxygen of 48 mm., which is equivalent to an approximate altitude of 30,000 feet.

The same altitude equivalents and length of exposure were used in testing effects of sulfapyridine on susceptibility to anoxia. One of 10 treated mice died when exposed to a pressure corresponding to an approximate altitude of 18,000 feet, but 20 treated mice survived exposure to pressures equivalent to approximate altitudes of 24,000 or 28,000 feet. One of 15 treated mice died during exposure to a partial pressure of oxygen of 48 mm. (30,000 feet).

Parallel experiments were made with rodilone, except that no mice were exposed to a partial pressure of oxygen of 100 mm. Three of 10 mice died during exposure to a pressure corresponding to an approximate altitude of 28,000 feet. During exposure to a pressure equivalent to an approximate altitude of 30,000 feet, however, none of 20 treated mice died.

Groups of 10 mice treated with aeacia alone were exposed to anoxia for one hour. One group was subjected to a partial pressure of oxygen of 100 mm., a second to a partial pressure of oxygen of 80 mm., a third to a partial pressure of oxygen of 64 mm., a fourth to a partial pressure of oxygen of 53 mm., and a fifth to a partial pressure of oxygen of 48 mm. In the last group a single death occurred.

Mice treated orally with 2.0 Gm. per kilogram of sulfanilamide showed marked prostration and slight convulsions. Degrees of anoxia used in this work had little effect on these symptoms. Mice treated with sulfanilamide were generally much quieter during the period of anoxia than were the control mice.

Treatment with 10.0 Gm. per kilogram of rodilone or sulfapyridine did not produce the prostration or convulsions observed in mice treated with sulfanilamide. Mice in these two groups were indistinguishable from the controls. It is notable that in these groups deaths occurred during anoxia, while no deaths occurred during anoxia in mice treated with sulfanilamide.

CUMULATIVE EXPERIMENTS

The three drugs and the vehicle were given orally, four times a day, for five days to four groups of mice before exposure to anoxia. Doses were spaced at approximately four-hour intervals, so that the mice received treatment for twelve hours each day. Since solution of these agents is slow, the actual time during which the mice were absorbing the drugs probably included at least sixteen hours each day. This procedure was adopted in order to follow as closely as possible the clinical use of these agents when intensive treatment is applied.

Also, single doses were low, although they were greater than the equivalent clinical doses on a weight basis. Doses used were below those which might be expected to produce any marked toxic symptoms. Sulfapyridine and rodilone were administered in single doses of 250 mg. per kilogram, corresponding to a daily dose of 1.0 Gm. per kilogram or a total dose of 5.0 Gm. per kilogram for the five days. Sulfanilamide was administered in single doses of 100 mg. per kilogram, corresponding to a daily dose of 400 mg. per kilogram or a total dose of 2.0 Gm. per kilogram for the five days.

All mice received these doses in single administrations of 0.1 ml. per 20 Gm. of solution, or a total of 100 ml. per kilogram of fluid in the five-day treatment period. The vehicle was 10 per cent acacia solution, and control mice received equivalent amounts of this. Since the mice voluntarily drank large amounts of water in addition to this, probably no disturbance in fluid balance occurred, although the daily amount of administered solution, 20 ml. per kilogram, is equivalent to the average daily urinary output in man. If hydration did occur, however, its possible effects on tolerance to anoxia are controlled, since the control mice received the same amounts of fluid.

Mice were weighed before the first treatment, on the third day of treatment, and on the sixth day, immediately before exposure to anoxia. The mean body weights of each group for these days were, respectively: for the controls, 21, 23, and 20 Gm.; for the group treated with sulfapyridine, 21, 23, and 20 Gm.; for the group treated with rodilone, 22, 22, and 20 Gm.; and for the group treated with sulfanilamide, 21, 20, and 19 Gm. Statistical examination of the standard errors of differences between these means revealed no significant changes. The slightly lower values on the sixth day were the result of a twelve-hour fast before exposure to anoxia.

RESULTS

On the sixth day, ten hours after the last treatment, the mice were exposed to anoxia for one hour. Twenty mice of each group were subjected to a pressure corresponding to an approximate altitude of 30,000 feet. One of the control mice which had been treated with acacia alone and 2 of the mice which had received rodilone died during anoxia. No deaths occurred during anoxia among mice which had been treated with sulfanilamide or sulfapyridine. At no time did any of the mice show the prostration or convulsions characteristic of acute sulfanilamide toxicity.

DISCUSSION

The results show that large oral doses of neither sulfanilamide nor sulfapyridine increase susceptibility to anoxia, nor do these agents exert a cumulative

effect which is detrimental to anoxic animals. This evidence might be expected from the success of the clinical use of these agents in pneumonia.

Results with rodilone are not sufficiently significant, when examined by the criteria of Loewenthal and Wilson,⁶ to permit the conclusion that rodilone has any effect on tolerance to anoxia. Molitor and Robinson⁴ comment on the marked cyanosis developed during repeated administration of rodilone. Although the careful studies of Harris and Michel,⁷ and Long, Bliss, and Feinstone,⁸ show the presence of methemoglobin or sulfmethemoglobin, or a fall in oxyhemoglobin content, in the blood of patients treated with sulfanilamide or sulfapyridine, it must be concluded from the present results that neither of these processes is important in mice treated intensively with any of the three compounds studied. Hematuria, if it occurred, was not sufficiently grave to produce an anemia severe enough to lower tolerance to anoxia.

The results may be considered confirmatory of Brun's⁹ finding that these drugs do not increase the metabolic rate. Certainly there is no evidence of increased need of oxygen in the treated mice.

Of more practical importance than the present toxicity studies is the possible effect of sulfanilamide-type compounds on functions involved in precise orientation of persons. Rook² and Robertson¹⁰ have indicated the possible dangers of allowing patients treated with sulfanilamide to engage in activities requiring precise judgment of orientation and speed. Experimental studies on man should be made to evaluate this point properly. In addition, the effect of the disease, for which sulfanilamide is being given, on judgment and coordination, must also be considered.

SUMMARY

Mice treated with single large oral doses of sulfanilamide, sulfapyridine, or rodilone showed no increased susceptibility to anoxia. Repeated treatment with smaller doses of these three agents given orally, four times a day, for 5 days also did not increase susceptibility to anoxia.

We are grateful to Dr. Hans Molitor, of the Merck Institute for Therapeutic Research, for generously supplying sulfapyridine (Dagenan) and rodilone (di-p-acetylamino-phenyl-sulfone). All rodilone used was of the same preparation, designated as lot 144.

REFERENCES

1. Mackie, F. P.: Sulphanilamide in the Air: A Warning, *Brit. M. J.* 2: 139, 1939.
2. Rook, A. F.: Sulphonamide in the Air, *Brit. M. J.* 2: 306, 1939.
3. Foreign Letter, *J. A. M. A.* 113: 1237, 1939.
4. Molitor, H., and Robinson, H.: The Acute, Cumulative and Chronic Toxicity of 2-Sulfanilyl Aminopyridine and di-(p-acetylamino-phenyl)-sulfone, *Arch. internat. de pharmacodyn. et de therap.* 62: 281, 1939.
5. Kolls, A. C., and Loevenhart, A. S.: A Respiratory Chamber for Small Animals, *Am. J. Physiol.* 39: 67, 1915.
6. Loewenthal, L. J. A., and Wilson, W. A.: Problems of Chance in Medicine and Research, *Brit. M. J.* 2: 110, 1939.
7. Harris, J. S., and Michel, H. O.: Formation of Hemoglobin and Sulfhemoglobin During Sulfanilamide Therapy, *J. Clin. Investigation* 18: 507, 1939.
8. Long, P. H., Bliss, E. A., and Feinstone, W. H.: Effects of Sulfapyridine, Sulfanilamide and Related Compounds in Bacterial Infections, *Pennsylvania M. J.* 42: 483, 1939.
9. Brun, G. C.: Gibt es eine Stoffwechselsteigerung nach Zufuhr von Sulfanilamiden? *Arch. internat. de pharmacodyn. et de therap.* 63: 103, 1939.
10. Robertson, D. F.: Personal communication, Nov. 7, 1939.

INFLUENCE OF SODIUM CHLORIDE CRYSTALLIZATION BY CEREBROSPINAL FLUID*

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FOLLOWING the evaporation of a salt solution there results crystal formation which is specific for each salt and is the so-called crystal habit of the salt. Various workers have shown that the crystal formation of a salt may be easily affected by many factors. The minutest impurity in the salt solution will influence the crystal habit. Semenenko and Shikhobalava¹ showed that the surface tension of the salt solution was the most important factor influencing the crystallization. Changes in the surface tension are reflected by changes in the crystal habit. These workers showed that the crystallization of sodium nitrate by evaporation is hardly influenced by substances which cannot change the surface tension of this salt. By the addition of surface-active substances a typical change of the crystal configuration is possible. Among the most important surface-active substances are albumin and globulin. These physicochemical facts were first used by Pfeiffer,² who noted that the addition of minute quantities of blood influenced the crystallization of a copper chloride solution. He further observed that blood from a tuberculous or cancerous patient gave a different type of crystal formation than did normal blood. He felt that by this method a serologic diagnosis of certain diseases was possible.

Tomeseo and his co-workers³ used the same principle for the study of the cerebrospinal fluid. They added minute quantities of cerebrospinal fluid to physiologic saline solution. They then studied the results of the crystallization by evaporation of this mixture at a temperature of 75° C.

The evaporation of 3 drops of physiologic saline solution results macroscopically in the formation of an outer ring surrounding an empty space. Microscopically, the sodium chloride crystals have a cubical shape. The addition of normal cerebrospinal fluid changes this picture. Tomeseo and his associates found that the normal cerebrospinal fluid is able to change the crystallization in a dilution of 1:20 up to 1:60 or 1:80. Macroscopically, the evaporation of 3 drops on a slide shows a crystalline circumference enclosing a translucent layer. With higher dilutions this translucent layer disappears, and one sees only empty spaces, i.e., the same picture as seen without the addition of cerebrospinal fluid. Microscopically, the translucent area consists of rosettes, concentric circles, parallel lines, and needles.

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In cases of general paresis Tomesco and his co-workers found typical changes in the crystal habit. Macroscopically, the central translucent layer was found to be present in dilutions up to 1:120. Microscopically, they found concentric circles, parallel lines, and fine needles, but the organization of the pattern was much finer than that of normal cerebrospinal fluid. The concentric circles around one or two cuboid sodium chloride crystals were especially finely organized. In all cases the cerebrospinal fluid showed positive Pandy and Nonne-Apelt tests, i.e., signs of increased protein content. In a later publication Tomesco and his collaborators stressed the fact that they found the so-called mixed form of crystallization in such mental diseases as schizophrenia and melancholia. This consisted of pictures which were between the normal and pathologic types. They felt that the increased protein is a very important factor but not the only one which can explain the reactions.

Finkelman⁴ used the technique of Tomesco in a large series of cases. He examined 35 specimens of cerebrospinal fluid of patients with general paresis and found typical pathologic crystal patterns in 33 specimens. Of 17 persons with functional epilepsy, 11 showed normal pattern and 6 were of the mixed type. Of 16 persons with dementia praecox, 10 were normal and 6 were of the mixed type; in all these patients the Ross-Jones test was negative. Seventy-one specimens of spinal fluid from persons with nonneurologic disorders were all negative.

Recently Selzer⁵ used Tomesco's technique but obtained different results. Although he found in the main in normal persons and in persons with pathologic disorders the same results as Tomesco, he is of the opinion that this method is without practical value because the formation of the pattern depends so much upon minute changes of the surface tension that sometimes drops from the same dilution evaporated on different slides, but at the same temperature, may show a different pattern formation. Selzer showed that there is an exact mathematical parallelism between the degree of the albumin content and the pattern formation.

The results obtained by these previous workers seemed of sufficient importance to us to prove the diagnostic value of this new reaction. In order to exclude any subjective factor the clinical diagnosis of the fluids examined was not disclosed to the examiner.

TECHNIQUE

We followed the method used by Tomesco and his co-workers and Finkelman. Briefly, this consisted of setting up ten test tubes of 0.8 per cent sodium chloride solution. The first tube contained 1 c.e., the second, 2 c.e., with increasing amounts up to 10 c.e. in the tenth tube. To each tube was added 1 drop of cerebrospinal fluid, and the tube was well agitated. Three drops of the contents of each tube were placed on separate slides, and the slides in turn were placed in an oven at 75° C., until the fluid was entirely evaporated. The slides, tubes, and pipettes used must be very clean and contain no impurities. The temperature is very important. We found that if the slides were kept at room temperature, or even up to 50° C., the crystallization shows a different picture.

The effect of other substances in influencing the crystal habit was shown by the following experiment: Using cerebrospinal fluid from a patient with general paresis, a typical organization pattern was obtained. To the same fluid in the test tube we now added 1 drop of an insulin solution; this led to the disappearance of the previous characteristic crystallization. The interpretation of the results is important. It seemed to us that the previous readings as positive, negative, or mixed type were not sufficiently exact. We also felt that the macroscopic picture had no diagnostic value in comparison to the microscopic picture. Normal or negative fluids show the following picture: Macroscopically, in a dilution of from 1:20 to 1:80 there is a translucent layer surrounded by a crystalline ring. This layer gradually disappears with higher dilutions. Microscopically, the translucent area consists of rosettes, concentric circles, parallel lines and needles (Fig. 1). In higher dilutions the pattern gradually disappears.

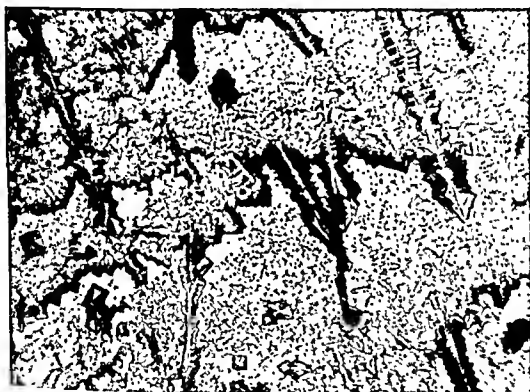


Fig. 1.—Crystallization of sodium chloride influenced by normal cerebrospinal fluid, dilution 1:80 (photomicrograph 1:80).

We divided the positive cases into three-, two-, and one-plus reactions. A three-plus fluid was characterized as follows: Macroscopically, there were no empty spaces in the translucent layer, even in a dilution of 1:200, i.e., 1 drop of spinal fluid plus 10 c.c. of saline solution. Microscopically, there were concentric circles, parallel lines, and fine needles, but the organization of the pattern was much finer and of a different nature (Fig. 2). The characteristic sign of a strongly positive pathologic fluid was the presence of a central cuboid crystal surrounded by many fine concentric rings. This is called good organization of the pattern and may be found in dilutions of 1:200 or even higher.

In a two-plus fluid the microscopic picture was the same qualitatively, but quantitatively there was a difference because the good organization was found up to only 1:140 or 1:160 dilution. The one-plus fluid shows a good organiza-

tion only in dilutions up to 1:100 or 1:120. As doubtful fluids we considered those cases where there was a more or less good organization up to 1:80. Sometimes one can find, even in normal spinal fluids in low dilution, a good organization pattern in the region just inside the peripheral crystalline ring. We do not consider this a pathologic finding.



Fig. 2.—Crystallization of sodium chloride influenced by cerebrospinal fluid from a case of general paresis, dilution 1:200 (photomicrograph 1:80).

MATERIAL

We examined 263 persons. This consisted of 151 persons with general paresis, 46 with dementia praecox, 26 with chronic alcoholism, 8 with idiopathic epilepsy, 8 with mental deficiency, 4 with psychoneuroses, one with multiple sclerosis, one with pachymeningitis hemorrhagica interna, one with manic-depressive insanity, 8 with cerebral arteriosclerosis, and 9 without psychosis.

General Paresis.—Many of the 151 patients had been treated for varying periods of time, and some had completely negative serologic and spinal fluid findings. Of these, 31 gave a three-plus crystallization; 38, a two-plus; 46, a one-plus; 2 gave a doubtful and 34 a negative crystallization. In other words, the crystallization was positive in 115, or 76 per cent, of the cases.

Of more importance is a comparison between the crystallization finding and the other laboratory findings.

1. Of the 31 persons with a three-plus crystallization the blood Kahn test was positive in 27, and the spinal fluid Wassermann test was positive in all. The Lange colloidal test gave a typical paretic curve in 22 persons, a middle zone curve in 8, and a negative result in 1. The Ross-Jones test was positive in 12 persons, gave a trace of globulin in 12, and was negative in 7; i.e., this test was negative in 22 per cent of strongly positive crystallizations. The cell count was above 10 in 15 cases.

2. Of the 38 persons with a two-plus crystallization the blood Kahn test was positive in 27, and the spinal fluid Wassermann test was positive in 36. The Lange colloidal test gave a typical parietic curve in 4 persons, a middle zone curve in 30, and a negative result in 4. The Ross-Jones test was positive in 2 persons, gave a trace of globulin in 16, and was negative in 20; i.e., this test was negative in 52 per cent of the cases. The cell count was above 10 in 14 cases.

3. Of the 46 persons with a one-plus crystallization the blood Kahn test was positive in 30, and the spinal fluid Wassermann test was positive in 42. The Lange colloidal test was typical in one person, gave a middle zone in 33, and was negative in 12. The Ross-Jones test was positive in 2 persons, gave a trace of globulin in 10, and was negative in 34; i.e., this test was negative in 73 per cent of the cases. The cell count was above 10 in only 7 cases.

4. One of the two persons with doubtful crystallization showed a positive blood Kahn test; both showed negative spinal fluid Wassermann tests and negative Lange and Ross-Jones tests. The cell count was not increased.

5. Of the 34 persons with negative crystallization the blood Kahn test was positive in 7. Both the spinal fluid Wassermann and the Lange tests were negative in every case; in only one case did the Ross-Jones test show a trace of globulin and only in this case was the cell count above 10.

6. Of the 115 persons with positive crystallization the Ross-Jones test was positive in only 54, or 46 per cent of the cases. The Lange test was positive in 98, or 85 per cent of the cases. The spinal fluid Wassermann test was positive in 108, or 93 per cent of the cases. The cell count was above 10 in only 36, or 31 per cent of the cases. These results seem to indicate that the results of the crystallization show a parallelism first to the spinal fluid Wassermann test, and second, to the Lange test.

7. The crystallization was positive in every case in which the spinal fluid Wassermann test was positive, and in every case in which the Lange test gave a typical or middle zone curve. On the other hand, there were other cases in which the crystallization was positive, but the Lange, Ross-Jones, and spinal fluid Wassermann tests were negative.

Dementia Praecox.—We examined 46 persons with various types of dementia praecox. The crystallization was positive in 7 persons, doubtful in 4 and negative in 35. In the 7 persons with positive crystallization the blood Kahn test was positive in every case, the spinal fluid Wassermann test was positive in 4, the Lange test was of the middle zone type in 2, and the Ross-Jones test showed a trace of globulin in one case. In the doubtful cases the blood Kahn was positive in one case. In each one of the 7 positive cases syphilis was associated with the dementia praecox.

Chronic Alcoholism.—Of the 26 persons with chronic alcoholism one gave a positive and one gave a doubtful crystallization. No sign of syphilis was noted in either person.

Epilepsy.—Of the 8 persons with epilepsy one showed a two-plus crystallization. In this person the blood Kahn and the spinal fluid Wassermann tests were positive, and the Lange test was typical.

Mental Deficiency.—Of the 8 persons with mental deficiency 2 gave a doubtful crystallization, both of whom had positive blood Kahn tests but negative spinal fluid tests.

Psychoneuroses.—Of the 4 persons with psychoneuroses one gave a positive and one a doubtful crystallization. The person with the positive crystallization showed negative blood and negative spinal fluid tests, but his history stated that he had been treated for syphilis. The patient with the doubtful reaction had a positive blood test.

Cerebral Arteriosclerosis.—Of the 8 persons with cerebral arteriosclerosis two had positive crystallizations and positive blood Kahn tests but negative spinal fluid tests.

Without Psychosis.—All the remaining patients gave negative results.

COMMENT

A review of the preceding problem brought up the question of the factor or factors involved in the crystallization. Tomesco and his co-workers at first felt that the albumin-globulin content of the spinal fluid was the important factor because they noted that these substances are able to change the surface tension of the solution. In a later publication they stated that this cannot be the only factor, since they found strong pathologic or mixed type crystallization in persons with normal cerebrospinal fluid. Selzer stressed the increased albumin content as the only responsible factor. To prove this he showed that by removing the albumin of the spinal fluid the remaining fluid had no influence on the crystallization. By removing the albumin from normal and pathologic spinal fluids, as in general paresis, we got the same results as Selzer. However, we do not feel that we should draw the same conclusion as he did, i.e., that the only factor involved is the increased albumin, for it is possible to eliminate other unknown factors by removing the albumin. Perhaps besides the quantitative increase of protein in the spinal fluid, certain qualitative changes of the proteins are important, or a change in the albumin-globulin ratio may be responsible. From a practical point of view our results differed somewhat from those of previous workers. In case of general paresis our results were similar. However, in those cases of dementia praecox where we obtained a positive crystallization, syphilis was present in almost every case. In an occasional instance, alcohol seemed to be the only predisposing factor. It was also interesting to note in cases of general paresis that the crystallization phenomena seemed to be more sensitive than the colloidal gold curve.

We feel that further studies are indicated to discover the factors responsible for the crystallization phenomena.

SUMMARY

We examined the effect of normal and pathologic cerebrospinal fluid on the crystallization of sodium chloride. Spinal fluid of general paresis influences the crystal habit of sodium chloride in a typical manner.

REFERENCES

1. Semenchenko, V. K., and Shikhobalava, L. P.: The Effect of Impurities on Crystallization, *Mineral Sui'v* 11: 27, 1936. (Chem. Abst. 30: 7014, 1936.)
2. Pfeiffer, E.: Empfindliche Kristallisationsvorgänge als Nachweis von Formungskraften im Blut, Dresden, 1935, Emil Weise.
3. Tomesco, P., Cosmulesco, I., and Serban, F.: Une propriété inconnue du liquide céphalo-rachidien: la chloruro-cristallisation. Les aspects normaux, *Bull. Soc. de psychiat. de Bucarest* 1: 11, 1936; La chloruro-cristallisation dans la paralysie générale. Les aspects pathologiques de cette propriété du liquide céphalo-rachidien, *Bull. Soc. de psychiat. de Bucarest* 1: 38, 1936; La chloruro-cristallisation dans les maladies mentales non syphilitiques, *Bull. Soc. de psychiat. de Bucarest* 1: 52, 1936; Formations cristallines du liquide céphalo-rachidien et chloruro-cristallisation, *Bull. Soc. de psychiat. de Bucarest* 2: 122, 1937.
4. Finkelman, Isadore: The Effect Upon Sodium Chloride Crystal Growth of Contamination With Normal and Abnormal Cerebrospinal Fluids, *J. LAB. & CLIN. MED.* 23: 1153, 1938.
5. Selzer, H.: Crystallization of Chlorides in Cerebrospinal Fluid, *Arch. f. Psychiat.* 107: 775, 1936.

100 E. JEFFERY STREET

OBSERVATIONS ON THE INTERRELATIONSHIP OF CAPILLARY, PLATELET, AND SPLENIC FACTORS IN THROMBOCYTOPENIC PURPURA*

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IN CASES of thrombocytopenic purpura a discrepancy between the degree of clinical bleeding and the level of the platelet count is not infrequently observed. One of us in a recent publication¹ was able to show that when this occurs the resistance of the capillaries, as measured by Dalldorf apparatus, tends to parallel the degree of clinical bleeding rather than the platelet level. In the same article attention was also called to the facts that following the performance of splenectomy for the relief of purpura, the capillary resistance rises with abrupt rapidity, that it often precedes in this respect the elevation of the platelet count, and that it may, therefore, be the first measurable sign of clinical improvement. In addition, it was concluded as a corollary that the suction test for capillary resistance was a useful method of measuring the activity of this disease and that in certain instances it was of more value in this connection than the enumeration of the blood platelets.

Since the publication of these investigations, we have been able to confirm the foregoing observations on numerous occasions. The number of consecutive cases of purpura which have been subjected to splenectomy and so studied in this clinic now totals 12. In every instance the results of our observations on capillary resistance have been essentially the same.

As a control to the latter observations, resistance determinations have been performed upon five individuals undergoing splenectomy for conditions

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other than purpura. In only one instance was any alteration in resistance noted, and this proved to be a slow rise, quite unlike those observed in the purpura cases, in a patient whose spleen was removed to facilitate the excision of a pancreatic adenoma. In this instance, the change may well have been due to the general improvement of the patient following the removal of the tumor.

As additional controls the capillary resistance was followed in five individuals undergoing relatively simple abdominal operations in which the spleen was not removed. In none of these patients were the resistance values altered significantly.

To explain the relatively common phenomenon of the discrepancy between the platelet level and the intensity of the hemorrhagic manifestations in purpura, it becomes necessary to assume the presence of both a capillary and platelet factor in the mechanism of the disease. Bedson² gives the credit for advancing this hypothesis to Pierre Nolf, who offered the following ingenious experimental evidence in its support:

Using rabbits as subjects, he produced a marked, but transitory, thrombocytopenia by the intravenous injection of agar, and found that no purpura resulted. He then damaged the capillary endothelium of other rabbits by the intravenous administration of an anti-rabbit red blood cell serum. Again no purpura resulted, despite the fact that actual injury to the capillary endothelium could be demonstrated microscopically. When, however, he combined the two procedures, administering to the same animal first the anti-red blood cell serum and then the agar, i.e., when he added a thrombocytopenia to capillary damage, a marked degree of purpura resulted.

Inasmuch as our clinical investigations seemed not only to support Nolf's theory but also to establish the suction test as a relatively accurate method of measuring the severity of the disease process in purpura, we decided to repeat Bedson's experiments, hoping that by following the resistance of the capillaries we could better evaluate the individual importance of the capillary and platelet elements in the mechanism of this disease. In addition, it also seemed desirable to investigate the relationship of the spleen to these two factors. Therefore, for the sake of completeness and because of certain advantages which will be apparent later, we adopted the antiplatelet serum method of producing purpura as an alternative to the technique of Bedson described in the preceding paragraph.

TECHNIQUE

We employed then two separate and distinct techniques in the production of experimental purpura. They were (1) the antiplatelet serum method and (2) the method of Bedson. A brief description of each method is presented. If more detailed information is desired, the papers of some of the many investigators who have used essentially similar techniques may be consulted.^{2,3} Light-skinned, male chinchilla rabbits, weighing between 2 and 3 kg., were used as the experimental animals throughout.

The Antiplatelet Serum Method.—In preparing the platelet antigen for the production of purpura by means of antiplatelet serum, the blood of two rabbits was drawn from the heart under nembutal anesthesia into an equal quantity of oxalated saline (sodium chloride 0.9 per cent and potassium oxalate 0.2 per cent). After centrifuging rapidly for one-half hour, the buffy coat was pipetted off and washed three or four times in normal salt solution to remove the remaining red blood cells as completely as possible. The yield, generally averaging between 1.5 and 2.5 c.c., was suspended in saline and injected intravenously into dogs. Four such injections were given at weekly intervals. A week after the last injection the dogs were bled and the desired quantity of antiplatelet serum was obtained. The serum so prepared was then injected intraperitoneally into rabbits, a dosage of 1.5 c.c. per kilogram of body weight being generally sufficient to produce a moderately severe thrombocytopenic purpura of from three to five days' duration (see Chart 1).

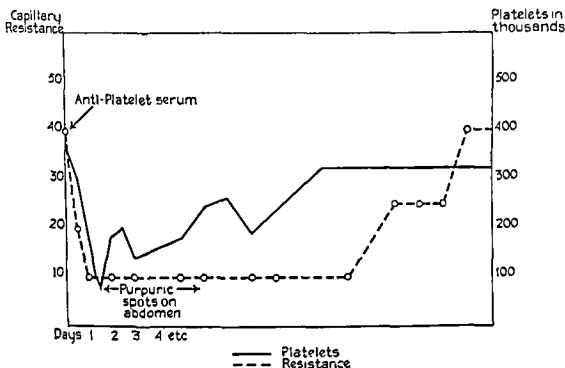


Chart 1.—Experimental thrombocytopenic purpura by means of antiplatelet serum showing the lag of the capillary resistance behind the platelets in returning to normal. Note also the relatively short duration of the purpura.

Bedson's Method.—The technique used in the preparation of an anti-red blood cell serum for the production of purpura by the previously outlined method of Bedson was essentially similar to that employed in the preparation of antiplatelet serum. At weekly intervals dogs were injected intravenously with a saline suspension of 2 c.c. of washed rabbit red blood cells, and a week after the fourth injection the desired amount of anti-red blood cell serum was obtained.

The agar-treated serum to be used in conjunction with the anti-red blood cell serum was made up as follows: One part of a suspension of 0.5 per cent agar in saline was mixed with four parts of fresh normal rabbit serum. The mixture was then incubated at 37.5° C. for two hours and allowed to stand at room temperature overnight. The following day it was centrifuged to remove any macroscopic agar and the clear supernatant serum was pipetted off. To produce purpura the anti-red blood cell serum was first injected intravenously into a rabbit, the dosage being 0.85 c.c. per kg. Three or four hours later the agar serum was administered to the same animal in similar fashion, the dosage in this instance being 5 c.c. per kilogram of body weight. The resulting purpura was to all appearances similar to that produced by antiplatelet serum, but more severe (Chart 2a).

OBSERVATIONS UPON NORMAL RABBITS

Fifty-six rabbits were used in the course of our investigative work. Upon 54 of these at least one or more control determinations were made. The determinations were carried out before subjecting the animals to experimental

procedures which would in any way affect their apparent good health. These determinations included hemoglobin values, red and white corpuscles, differential blood counts, bleeding and clotting times, clot retractility estimations, platelet counts, and capillary resistance determinations. This information is summarized in Table I, which includes the maximum, minimum, and mean values, together with the number of initial observations for each determination.

TABLE I
CONTROL VALUES

	NO. OF INITIAL OBSERVATIONS	MAXIMUM	MINIMUM	MEAN
Hemoglobin*	43	90	71	79
Red blood cell count	44	6,900,000	5,000,000	6,000,000
White blood cell count	42	12,100	5,600	9,100
Differential count†				
Polymorphonuclears	40	71	26	49
Lymphocytes	40	66	18	42
Monocytes	40	8	0	4
Basophiles	40	10	0	2
Eosinophiles	40	6	0	3
Bleeding time‡	41	4'00"	1'10"	2'14"
Clotting time	40	3'00"	0'45"	1'41"
Clot retractility§	22	24+ hr.	1½ hr.	
Platelets¶	54	438,000	178,000	298,000
Capillary resistance	33	50 plus	15	

*Sahl.

†Figures given in per cent.

‡Duke's method.

§In 7 cases there was no retraction after twenty-four hours.

¶Direct method.

||Figures given are in centimeters of mercury negative pressure.

The figures in Table I for the hemoglobin content and cellular elements of the blood agree closely with those of the majority of other observers who have employed the same technical methods as were used here. Determinations of the bleeding time were not always satisfactory, as the test was done by pricking the marginal ear vein, the state of contractility of which was found to influence markedly the results in several instances. It was likewise found that estimations of clot retractility on the normal animal were of little use as control observations, for in a third of the animals upon which the test was performed, retraction of the clot was either delayed or failed to take place at all.

As in our clinical work, all capillary resistance determinations were done with the Dalldorf apparatus. The animals were shaved prior to testing, and the tests were carried out on the skin of the abdominal wall, the technique used being identical in every respect with that employed clinically.

The normal figures for capillary resistance in the rabbit were slightly higher than those we observed in human beings. In man we have found it ranged between 20 and 35 cm. of mercury negative pressure when taken on the forearm just below the antecubital fossa;¹ in the rabbit it ranged between 30 and 40 cm. of mercury negative pressure, with a mean value of approximately 35. The variation observed between rabbits tended to be greater than the variation between human beings, though an effort was made to minimize this discrepancy as far as possible by selecting light-skinned animals of the same

breed. The reason for this greater variation is due to the fact that greater individual differences exist in the texture, color, and thickness of the skin among rabbits than among human beings.

Two other factors that tended to influence the results of resistance determinations were shaving and the application of antiseptic solutions to the skin of the abdominal wall immediately prior to operative procedures. For this reason, when shaving was indicated, it was carried out twenty-four hours in advance of the performance of any determinations and iodine was generally omitted from the preoperative preparation of the skin, or if it was used, it was applied in weak dilutions, care being taken subsequently to remove it completely.

Despite the number of factors capable of influencing the results of this test, there was little variation between readings taken on different areas of the abdominal wall in the same animal, provided that the test was not performed on the flanks or in the groin.

Undoubtedly the suction test for capillary resistance is not as accurate in rabbits as it is in human beings. For this reason, we do not regard a change of resistance in rabbits as being of much significance unless it is greater than five points. Notwithstanding this, we feel that within broad general limits the test does give a reasonably accurate estimation of the state and change of resistance in the capillaries of the animals under discussion.

PROCEDURES

In our attempt to study experimentally the mechanism of the disease process in purpura, we pursued our investigations along lines as nearly parallel with our clinical studies as possible. With this in mind, the thrombocytopenic purpura produced by antiplatelet serum and Bedson's anti-red blood cell serum-agar serum methods was studied first. Subsequently, the effect of splenectomy both before and after the production of purpura by these methods was investigated.

PRODUCTION OF PURPURA

No difficulty was experienced in the production of purpura by means of either technique used. It was found that following the employment of these methods a thrombocytopenic purpura was produced which resembled the clinical form in every respect save that of duration, the experimental syndrome lasting usually no longer than five days. Of considerable interest was the fact that the capillary resistance which dropped along with the platelets generally required a much longer time to return to normal levels than did the latter (Chart 1).

Like Bedson, we found that the injection of anti-red blood cell serum alone, or of agar serum alone, did not produce purpura. In addition, these procedures had no appreciable effect upon the capillary resistance. We found, furthermore, that as a whole the animals subjected to Bedson's method developed a more severe grade of purpura than did those injected with anti-platelet serum. Charts 1 and 2a depict graphically the typical course of events in animals subjected to both of the afore-mentioned techniques.

SPLENECTOMY AND EXPERIMENTAL PURPURA

A. Operative Controls.—In order to parallel our clinical studies on splenectomy and to establish control groups for similar experimental investigations, the effect both of simple laparotomy and of splenectomy upon the blood values and the capillary resistance of normal animals was investigated. Ether anesthesia was employed in both procedures and the operative technique in each was identical, the only difference between the two operations being that in one the spleen was removed, and in the other it was exposed but not disturbed.

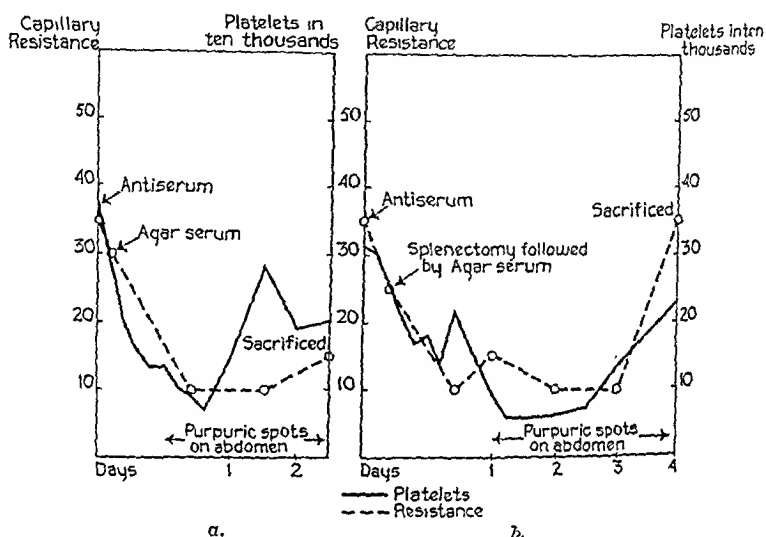


Chart 2a.—Experimental thrombocytopenic purpura by means of the combined administration of antiserum and agar serum. At autopsy this animal showed a very severe purpura with abundant hemorrhages into the mesenteries, the pancreas, and the subserosal tissues of the intestines. *b*, Experimental thrombocytopenic purpura following splenectomy performed during the combined administration of agar serum and antiserum. Autopsy showed severe purpura.

Following the performance of simple laparotomy, a slight rise in platelets was observed in each animal, the maximum and minimum increments for three animals thus followed being 96,000 and 19,000, respectively. The increments in the latter values following removal of the spleen, however, were considerably greater and averaged between 100,000 and 200,000 (see Chart 3b). In neither of these control procedures was the capillary resistance altered significantly.

B. Protective Effect of Splenectomy.—Bedson⁷ has found that in guinea pigs splenectomy affords a varying degree of protection against the purpura produced by antiplatelet serum when this operation is performed prior to the administration of the serum. Because we wished to determine whether the same results obtained in rabbits and whether the performance of splenectomy before the injection of antiplatelet serum had any effect upon the capillary resistance, we repeated Bedson's experiment upon three animals.

Accordingly, the animals' spleens were removed, and six or seven days later, during the course of the ensuing platelet rise, antiplatelet serum was administered intraperitoneally. Despite the fact that the dose of the latter

was increased over the usual amount in all the animals and even administered in double the customary quantity in one, none of the rabbits developed purpura and none showed any change in capillary resistance (Chart 3b).

C. Splenectomy Following the Injection of Antiplatelet Serum.—The object of this group of experiments was to determine whether the removal of the spleen was as beneficial in experimental purpura as in the clinical form of the disease. Because of the relatively short duration of the purpura produced by the injection of antiplatelet serum, it was decided that it would be necessary to perform splenectomy early in the course of the disease. Therefore, as soon as a material drop in the platelet level and capillary resistance had occurred following the injection of the serum, the spleen of the animals was removed. It was realized that this was hardly a fair parallel to the circumstances obtaining when this operation is performed for the relief of purpura in man, and yet, for the reasons mentioned above, we could hardly do otherwise.

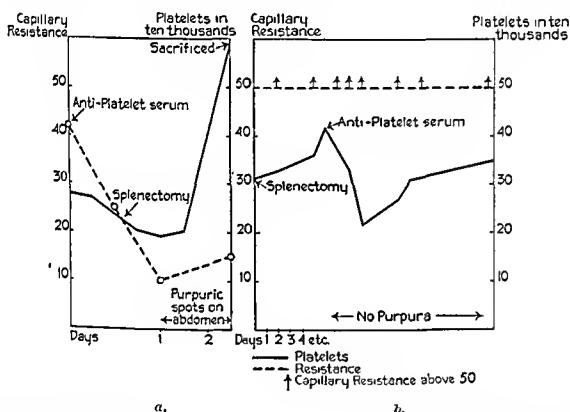


Chart 3a.—Experimental effect of splenectomy performed after injection of antiplatelet serum.

Chart 3b.—Protective effect of antiplatelet serum following splenectomy performed after moderate purpura.

Four animals, then, were injected intraperitoneally with antiplatelet serum. Between two and three hours later splenectomy was performed, for it was generally found that material depressions in both capillary resistance and platelet values had occurred within this space of time.

In each of the 4 animals so treated, a purpura of varying intensity developed, despite the fact that in none did the platelet level drop below 170,000. The findings were corroborated at autopsy on each of the animals. As will be seen in Chart 3a, a fall in capillary resistance was produced which lagged well behind the platelets in returning to normal.

D. Splenectomy and Experimental Purpura Produced by Bedson's Method.—In these experiments the decision as to where in the course of the procedure to intercalate splenectomy was particularly difficult. In the first place, the

same handicap existed here as was met with in the preceding experiment with regard to the brevity of the purpura. In the second place, it was obvious from earlier experiments that if the operation was performed immediately following the administration of agar serum, the animals would in all probability not survive the trauma incident to operation, together with the rapid development of a severe purpura, if such should occur. Therefore, it was felt that if the operation were performed shortly *before* the injection of the agar serum, but sufficiently long *after* the administration of the anti-red blood cell serum to allow the latter to affect the capillaries, the animals would have a better chance of survival. In addition, by this procedure we would be able to determine whether splenectomy negated to any extent the capillary-damaging effect of the anti-red blood cell serum.

Accordingly, splenectomy was performed on three rabbits between two and three hours after the administration of the anti-red blood cell serum. As soon as the animals had recovered from the effects of the anesthesia, agar serum was injected.

Severe purpura developed in all the animals (see Chart 2b). Not only did splenectomy fail to alter the effectiveness of the anti-red blood cell serum, but as nearly as could be determined it in no way lessened the severity of the resulting thrombocytopenic purpura, the presence of which was confirmed at post mortem on each of the animals.

COMMENT

From the foregoing investigations it seems apparent that the administration to rabbits of either antiplatelet serum, or of agar serum in combination with anti-red blood cell serum, is an eminently satisfactory method of producing in these animals a thrombocytopenic purpura which closely resembles the disease as seen clinically. It is worth emphasizing, however, that the experimental purpura so produced is of short duration and, to all appearances, only in this respect does it differ from the clinical syndrome. In producing purpura by the combined administration of anti-red blood cell serum and agar serum, we were able to confirm Bedson's results with this method, which, it will be remembered, were offered as evidence in support of Nolf's theory that both a capillary and a platelet factor are present in the mechanism of this disease and are necessary to its production.

From the results of Bedson's work and our own, it seems apparent that when either a capillary-damaging factor in the form of an anti-red blood cell serum, or a platelet-reducing factor in the form of an agar serum acts alone, it is incapable of producing purpura. It is equally evident, however, that when these two factors act together in the same animal, purpura results. Furthermore, from our investigations we have been able to show that, acting alone, neither one of these two factors materially alters the resistance of the capillaries, but that acting collectively, they do.

We have shown that splenectomy in rabbits neither affects the individual potency of the afore-mentioned capillary-damaging and platelet-reducing agents.

nor alters their combined efficacy in the production of purpura. Furthermore, while we have confirmed and extended to rabbits Bedson's results on the protective effect of splenectomy performed prior to the administration of antiplatelet serum, we have, on the other hand, also been able to show that the performance of this operation following the administration of this serum affords little or no protection against the subsequent development of purpura.

In addition, our experiments have demonstrated that simple laparotomy and splenectomy in the normal rabbit do not affect the resistance of the capillaries, but do cause an increase in the number of platelets, which is of considerably greater extent in the latter operation than in the former. These observations lead us to feel that, as intimated by Bedson, the protective effect of splenectomy performed prior to the injection of antiplatelet serum is purely an indirect one and is due solely to the physiologic increase in the number of platelets which follows this operation. Additional confirmation of this would seem to lie in the fact that following the production of thrombocytopenic purpura by either the antiplatelet serum method or the agar serum method, and irrespective of whether the spleen was removed or not, the resistance of the capillaries undergoes a marked drop and in most instances does not return to its original value until well after the platelet count has resumed its normal level.

DISCUSSION

Though the thrombocytopenic purpura produced by the serologic methods herein described closely resembles in its manifestations the clinical form of this disease, there is no justification for assuming that the role of the spleen in the experimental syndrome is anything other than a minor one and of secondary importance. Certainly our experimental work, as well as that of Bedson, would seem to argue against such an assumption. On the other hand, the almost instantaneous rise in capillary resistance and the cessation of bleeding which follows removal of the spleen in idiopathic thrombocytopenic purpura is evidence that in the clinical form of the disease the role of the spleen is primarily an active one and of major importance. Further evidence for this contention is to be found in the fact that the afore-mentioned increase in the resistance of the capillaries and the alleviation of the hemorrhagic symptoms takes place too soon after operation to be accounted for by the subsequent increase in the number of platelets.

From our clinical as well as from our experimental work, we feel that we have definite evidence of the existence of both capillary and platelet factors in the mechanism of the disease process in thrombocytopenic purpura. In addition, we believe from our observations that the decrease in the resistance of the capillaries seen in this disease is probably the result of the combined action of these two factors. The fact that the capillary resistance tends at times to be dissociated from the platelet level makes us feel that the capillary factor is probably the more important of these two elements, if not in the initiation of the manifestations of the disease, at least in their maintenance.

SUMMARY AND CONCLUSIONS

1. A study of experimental thrombocytopenic purpura produced in rabbits by serologic methods has been made.
2. The relationship of splenectomy to the bleeding tendency, capillary resistance, and platelet count in thrombocytopenic purpura has been discussed.
3. In the absence of purpura, removal of the spleen in man and in rabbits does not affect the capillary resistance.
4. The suction method of measuring capillary resistance is a useful means of estimating the activity of the disease process in experimental as well as in clinical thrombocytopenic purpura.
5. It seems likely that both a platelet-reducing and a capillary-damaging element are present in the mechanism of the disease process in thrombocytopenic purpura and that both are necessary to the production of the disease.
6. The decrease in the resistance of the capillaries, which is seen in thrombocytopenic purpura, is probably the result of the combined action of the platelet and capillary factors.
7. Particular attention is directed to the importance of the capillary factor in thrombocytopenic purpura.
8. In experimental thrombocytopenic purpura produced by serologic methods, the role of the spleen is probably of secondary importance.
9. Evidence is advanced to support the contention that in idiopathic thrombocytopenic purpura the role played by the spleen is an active one and of major importance.

REFERENCES

1. Elliott, R. H. Egerton: The Use of the Suction Test for Capillary Resistance in Thrombocytopenic Purpura, *J. A. M. A.* 110: 1177, 1938.
2. Bedson, S. P.: Blood-Platelet Antiserum. Its Specificity and Role in the Experimental Production of Purpura. Part II, *J. Path. & Bact.* 25: 94, 1922.
3. Ledingham, J. C. G.: The Experimental Production of Purpura in Animals by the Introduction of Anti-Blood-Plate Sera; A Preliminary Communication, *Lancet*, June, p. 1673, 1914.
4. Ledingham, J. C. G., and Bedson, S. P.: Experimental Purpura, *Lancet*, Feb., p. 311, 1915.
5. Toeantins, Leandro M.: Experimental Thrombopenic Purpura in the Dog, *Arch. Path.* 21: 69, 1936.
6. Dalldorf, G.: A Criterion of Hemorrhagic Diathesis in Experimental Scurvy, *J. Exper. Med.* 53: 289, 1931.
7. Bedson, S. P.: The Effect of Splenectomy on the Production of Experimental Purpura, *Lancet*, Nov., p. 1117, 1924.

HISTAMINE THERAPY IN ALLERGY

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A REVIEW of the literature relative to the use of histamine in the treatment of allergic disturbances indicates that this substance is perhaps one of the best available agents for alleviating the discomfort caused by these disorders, particularly skin conditions characterized by wheal and edema formation, erythema, pruritus, and the exudative diathesis. Recently, the role of allergy in peptic ulcer has been considered, and certain cases have been found to be associated with allergic manifestations. In this paper the therapeutic effect of histamine in urticaria and other skin lesions and in gastrointestinal ulceration will be evaluated.

Histamine was found in putrid soybeans by Yoshimura³ in 1909, and was isolated from ergot in 1910 by Dale and Barger,³ who also found it to be present in the intestinal mucosa. Chemically, histamine results from the decarboxylation of histidine, and it may be formed by (1) chemical decarboxylation, (2) bacterial decomposition, (3) ultraviolet radiation, and from (4) di-amino-acetone by chemical synthesis. Histidine is widely distributed in the body, being most abundant in such tissues as the intestines, liver, and lungs.³

In 1911 Dale and Laidlow observed that histamine may cause the typical symptoms and pathology of anaphylaxis when injected into normal animals. It caused a drop in blood pressure when injected intravenously into dogs, and it produced an increased capillary permeability or an edema. They showed, however, that the reaction of histamine differs from true anaphylaxis, in that histamine is still capable of producing strong reactions in the uterine strip even though the latter has been desensitized by treatment with specific serum. Lumière and Malespine⁹ showed that the phenomenon of reciprocal protection from one shock by another shock, or anaphylaxis, extended equally to histamine accidents, suggesting an analogy in the mechanism of the two types of shock. These and other experiments revealed that histamine could cause in animals anaphylactic-like reactions which closely resembled allergic reactions, and the assumption was made that the formation of a histamine-like substance constituted a part of every allergic reaction.

In 1909 Eustis advanced a theory that asthma was produced endogenously by the absorption of a powerful toxin probably from the intestinal tract, and he suggested that histidine, or its histamine derivative, was so absorbed. Duke expressed the similar belief that allergic reactions may be caused by the absorption of a histamine-like substance from the intestines. Koessler³ found, however, that histamine was rendered inert by its passage through the intestinal mucosa, and Wangenstein and Loucks²² reported that the amount of histamine absorbed from a strangulated intestinal loop was very small.

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Making practical application of his theory, Eustis in 1912 began using histamine in small doses in the treatment of asthma. He reported beneficial results but stated that following injections of histamine several of his patients developed hives or nettle rash. This point is of signal interest in light of the more recent investigations of McConnell⁹ and her associates who found that histamine, when applied to the skin of animals, or of man, promptly produced a wheal formation similar to that elicited by ordinary allergens in positive skin reactions. Lewis^{3, 9} also has observed, in a study of the response of the skin and its blood vessels to a variety of stimuli, a reaction characterized by (1) local dilatation of minute blood vessels, (2) increased permeability of vessel walls by direct action, and (3) widespread dilatation of the neighboring arterioles through the medium of local reflex mechanism. He has shown that a similar reaction in the skin is produced by histamine, and he attributes this reaction to the effect of some chemical substance already present in the cells and perhaps released by the stimulus of histamine. In view of the histamine-like action of this substance, Lewis designated it the H-substance.

Kline, Cohen, and Rudolph,⁹ in an investigation to determine the cellular reaction in allergic and nonallergic patients, found that specimens of tissue from allergic individuals excised after injections of allergens and histamine, and exposure to heat, cold, and scratching, showed almost complete infiltration by eosinophilic cells, whereas the reactions of nonallergic tissue were minimal. The histamine wheals in allergic persons were comparable histologically to those produced by allergens. This study indicates a similarity in the reactions of allergic individuals to ordinary allergens and to histamine.

It is apparent that the skin response to histamine application resembles that of allergen inoculation, and both in turn are similar to the wheal, erythema, and pruritus found in the lesion of urticaria or hives and in the bite or sting of bees.^{19, 24} Apparently histamine or Lewis' histamine-like "H-substance" is present in all tissue cells, and, when reflexly stimulated, it acts by means of, or in conjunction with, certain as yet undetermined other substances to produce vascular disturbances. On the basis of this theory, the vascular response, according to its location, may be productive of hay fever, asthma, nettle rash, migraine, etc., including urticaria and other skin lesions, as well as peptic ulcer. Because of the histamine-like nature of the H-substance it appears reasonable to assume that repeated, small, increasing doses of histamine might reduce the tendency to vascular reactions (1) by acting as an antigen in conjunction with the tissue cells, (2) by desensitization, (3) by altering the chemical nature of the H-substance, or (4) by increasing the output of adrenalin or histaminase in the circulation.

The therapeutic efficacy of histamine in allergic disorders has been demonstrated by various investigators, who have reported its favorable use in asthma, migraine, lupus, pruritus, eczema, hypersensitivity to cold and to heat, periodic edema of the hand and angioneurotic edema, purpura, and other conditions including urticaria.^{1-8, 10-13, 16, 18, 21, 25-27}

I. TREATMENT OF URTICARIA AND OTHER ALLERGIC DISORDERS

My use of histamine in 53 cases of urticaria and other skin lesions was stimulated by the report of Dzsinich,⁴ who in 1935 reported complete relief in 12 of 15 cases of bronchial asthma and in 3 cases of urticaria after treatment with small doses of histamine. Following in general his outline of treatment, dosage beginning with 0.00001 mg. of histamine and increasing to 0.1 mg. was used, dilutions being prepared from 1 c.c. ampoules containing 1 mg., of histamine dihydrochloride (1:1,000 solution).⁶ The initial dose was given intradermally and, as a rule, was 0.00001 mg. of histamine. If no reaction or aggravation of symptoms occurred following this dose, the same amount was injected subcutaneously forty-eight hours later, and the dose was increased according to schedule, doses being given not oftener than every other day. Any dose that appeared to aggravate symptoms was promptly reduced to one-tenth of the amount before increasing in accordance with schedule. (Dosages for treating urticaria and allied lesions in infants and children should be reduced in proportion.)

The solutions of histamine were prepared as follows.

1. Take 0.5 c.c. of the ampoule solution and dilute to 5 c.c. with normal saline. This gives 5 c.c. of solution containing 0.1 mg. of histamine per cubic centimeter.

2. Make subsequent dilutions similarly to obtain solutions containing 0.01 mg. per cubic centimeter, 0.001 mg. per cubic centimeter, and 0.0001 mg. per cubic centimeter, i.e., 0.5 c.c. of each immediately stronger solution, diluted to 5 c.c. with normal saline, will give the next weaker concentration, or one-tenth that of the respective "mother" solution. Solutions should be placed in appropriately labeled vials.

Each individual should first be tested with 0.1 c.c. from the vial labeled 0.0001 mg. per cubic centimeter, and the following considerations as to dosage should apply:

(a) Histamine-sensitive persons may experience headache, flushing of the face, and slight aggravation of symptoms. For this classification, the vial containing 0.0001 mg. per cubic centimeter should be further diluted (*0.5 c.c. diluted with normal saline to 5 c.c. and labeled 0.00001 mg. per cubic centimeter*), and graduated doses of 0.1 c.c., 0.3 c.c., 0.5 c.c., and 0.8 c.c. should be given from this dilution before proceeding with the routine schedule.

(b) All other cases should follow the schedule as given.

(c) For infants and children, test with 0.0000001 mg. or 0.000001 mg., making necessary dilutions similarly to those above.

Histamine was injected in most cases according to the following schedule, given at forty-eight-hour intervals for an average total of eighteen injections.

Vial 0.0001 mg./c.c.

0.1 c.c. (0.00001 mg.) intradermally

0.1 c.c. (0.00001 mg.) subcutaneously

0.3 c.c. (0.00003 mg.) subcutaneously

⁶The histamine preparation used in this study was Imido "Roche," manufactured by Hoffmann-La Roche, Inc., Nutley, N. J.

	0.5 c.c. (0.00005 mg.) subcutaneously
	0.8 c.c. (0.00008 mg.) subcutaneously
Vial 0.001 mg./c.c.	0.1 c.c. (0.0001 mg.) subcutaneously
	0.3 c.c. (0.0003 mg.) subcutaneously
	0.5 c.c. (0.0005 mg.) subcutaneously
	0.8 c.c. (0.0008 mg.) subcutaneously
Vial 0.01 mg./c.c.	0.1 c.c. (0.001 mg.) subcutaneously
	0.3 c.c. (0.003 mg.) subcutaneously
	0.5 c.c. (0.005 mg.) subcutaneously
	0.8 c.c. (0.008 mg.) subcutaneously
Vial 0.1 mg./c.c.	0.1 c.c. (0.01 mg.) subcutaneously
	0.3 c.c. (0.03 mg.) subcutaneously
	0.5 c.c. (0.05 mg.) subcutaneously
	0.8 c.c. (0.08 mg.) subcutaneously
	1.0 c.c. (0.1 mg.) subcutaneously

Patients notice a sensation of coolness in the skin following the first injection, and blanching of the skin is caused by peripheral capillary constriction. This is followed, within a few minutes, by diminution of edema, disappearance of wheals, and diminution of pruritic symptoms, with complete remission within a few hours, as a rule. However, symptoms return in about forty-eight hours after the first dose, although to a lesser extent. Subsequent doses apparently desensitize or increase the tolerance of patients; the result is complete and lasting remission of symptoms in the most responsive cases. The responsive patient suffering from urticaria with edema is comfortable after 3 doses, without recurrence of lesions.

My series of 53 patients with allergic skin with urticarial manifestations included 30 of urticaria alone, 22 of urticaria and angioneurotic edema, and one of pruritus without urticaria. Forty of these patients, or 75.5 per cent, were completely relieved by histamine without any additional therapy. Eleven persons, or 20.7 per cent, were improved, but other measures, including acid and enzymé (pancreas) administration, autohemotherapy, diet, removal of infection, etc., were necessary to complete relief. Two, or 3.8 per cent, were not benefited; one patient was aggravated by even minute doses of histamine, and the other had a shock reaction and was finally relieved by hysterectomy and autohemotherapy.

The duration of urticaria in these patients was from a few days to seven years, the average being four weeks to six months. The refractory period obtained through histamine therapy has lasted from twelve to twenty-two months. Only 3 patients have required additional therapy after twelve months, with ensuing benefit.

Other allergic syndromes in which histamine was employed included 5 cases of asthma, 2 of vasomotor rhinitis, 3 of infantile eczema, 3 of senile eczema, one of acute localized eczema of the forearms, three of atopic dermatitis, 3 of pollen dermatitis, 2 of pruritus ani, one of simple pruritus vulvae with edema, 2 of pruritus vulvae with leucorrhœa, one of atrophic arthritis of the wrist, one of edema of the penis from ant bite, and one of hydroarthrosis of the knee, intermittent type.

SIDE REACTIONS

While small doses of histamine favorably influence the allergic disorder, larger doses aggravate paroxysms of asthma and also increase the intensity of urticarial symptoms. Final doses should not exceed 0.1 mg. or 0.1 c.c. of the original 1:1,000 solution. Toxic symptoms include headache, dizziness, profound weakness, flushing of the face to cyanosis, irritability of the nervous system, and dyspnea. Epinephrine is the natural antagonist of histamine and should be given if a severe reaction develops in any abnormally sensitive person.

Evidence of reaction may follow injection within a few minutes to several hours. In certain individuals a slight chilliness was experienced within fifteen to thirty minutes after injections, followed by headache, flushing of the face, drowsiness, nervousness, shortness of breath, and extreme weakness, lasting from a few minutes to, in one case, four days, during which time the patient did not feel able to get out of bed. As Brown has suggested, certain individuals manifest abnormal reactions to histamine; evidently after shock reactions a refractory period may follow.

In cases showing more than a mild reaction, subsequent doses should be reduced by one-tenth the original amount, given as mentioned previously. As a rule, untoward reactions do not follow reduction in dosage; instead there is definite improvement in symptoms. This being the case, histamine may then be given in increasing doses without fear of untoward accident. It has been noted that while initial minute doses may cause marked reactions in some cases, the tolerance of these patients apparently can be increased until large doses (approximately 0.1 mg.) produce no discomfort.

However, in view of the occurrence of a limited number of so-called toxic manifestations from small doses (0.00001 mg.), I feel it necessary to issue a word of caution against the indiscriminate use of histamine. It is apparently contraindicated in the presence of cardiac pathology, particularly in cases of myocarditis or coronary sclerosis.

CASE REPORTS

These selected case reports are representative of the favorable response to histamine therapy in urticarial lesions:

CASE 1.—R. A., 26-year-old male, a manufacturer of overalls, had had severe urticaria and sinusitis following undulant fever seven years previously. There was a history of allergy in his father's family. Both tonsils and adenoids had been removed. He had been a heavy drinker and stated that he had had urticaria almost constantly, especially after drinking. Physical examination was essentially negative. He gave positive skin tests to corn meal, rice, rye, cocoa, milk, chicken, asparagus, yeast, hops, all shellfish, and grass pollen (spring group). Treatment was begun with grass pollen desensitization and elimination diet. Sinusitis was relieved, and hay fever symptoms subsided, but urticaria was not benefited. Histamine therapy was begun, and, disregarding food tests, a full diet was given to combat a drop in weight. Doses from 0.000001 mg. increased to 0.5 mg. according to the outline of Draitch. Results were complete relief of urticaria, restoration of weight, stabilization of nervous system, and increase of energy. Six months later the patient once again began to take large quantities of alcoholic beverages without recurrence of urticaria except for one day. This occurred following a week's spree and was relieved by 0.00001 mg. of histamine intradermally.

CASE 2.—T. L., 31-year-old male, a railroad office assistant, had edema of the glottis, with generalized urticaria. He gave positive tests for a variety of foods and other substances and was subject to tingling of hands, face, and lower lip, followed by choking cyanosis and general urticaria. The first attack followed influenza. Physical examination was negative. He was given Rowe's diet for six months; his symptoms disappeared, and he was retested. His diet was changed; remission of symptoms followed for four months. A recurrent attack was relieved with 0.000001 mg. of histamine. The patient was desensitized with histamine and put on a regular, unlimited diet. There was no recurrence after four months, and the patient is in excellent health.

CASE 3.—F. W. G., male, an electrician, had acute urticaria and rhinitis of several weeks' duration. The patient's entire back was covered with brawny edema in which hard, marblelike areas could be palpated. Edematous area approximated 1 inch in thickness. Extremities were covered with macular-type urticaria with intense pruritus. Nasal discharge was clear but resembled that of hay fever in character. Moderate bronchitis was present. Treatment with small increasing doses of histamine was begun; total number of doses was 19. This was followed immediately by a gradual diminution of edema and disappearance of lesions. Body was clear within ten days. The patient has had complete relief for the past twenty-two months.

The foregoing case is typical of the majority of cases of urticaria relieved by histamine.

CASE 4.—T. T., male infant, aged 5 months, had acute infantile eczema of face, arms, and legs, and of body on ventral surfaces. There was a family history of allergy; the father had hay fever. The mother's milk did not agree with the patient so he had been fed with cow's milk formula since he was 2 months old. *No change in formula was made.* Histamine therapy was begun with a dose of 0.0000001 mg.; total number of injections was 11. Eczema disappeared in approximately five weeks. There was no recurrence within seventeen months.

This case is representative of the use of histamine in infants; similar decreased doses should be employed in other such cases.

CASE 5.—W. R. G., female, had severe pruritus ani and a history of hay fever, backache, and urticaria. Local examination by a proctologist revealed no pathology. Histamine was used for relief of intense pruritus; no local treatment was given to the rectum or the anus. A total of 19 injections, ranging from 0.000001 mg. to 0.1 mg. per dose, was given. This was followed by complete relief of itching symptoms and discomfort for thirty days, after which time symptoms returned.

Although relief in this case apparently was only temporary, it indicates the therapeutic value of histamine in this type of condition.

CASE 6.—R. E. B., female, for three years had had hay fever, asthma, and eczema (dry type) over the entire body as well as a tendency to angioneurotic edema and urticaria. She gave positive skin reactions to various foods and other substances. Diets and desensitization therapy were given for twelve months, with only incomplete relief from hay fever and recurrences of urticaria and eczema. Then histamine was given in doses of 0.00001 mg. intradermally to 0.1 mg. subcutaneously. As a result the skin is clear and apparently normal for the first time, and the patient notices only a slight early morning cough. Further treatment for pollen sensitivity is being given at present following completion of histamine therapy.

CASE 7.—M. M., school girl, had had itching for four months. Examination revealed scabies-like lesions with furunculosis from scratching and infection. Scabicides were used without relief. Intradermal histamine injection relieved itching within forty-eight hours, and histamine desensitization treatment was given. The result was complete relief.

DISCUSSION

The favorable results obtained in this series of cases bear out the reported effectiveness of histamine therapy in allergic manifestations. Use of histamine

in accordance with the desensitization schedule described would seem to offer a simple and reliable means of relieving skin disorders, in particular those disorders occurring as the result of an allergic reaction to external or internal irritants.

The lasting relief of conditions like urticaria afforded by a single series of histamine injections suggests that the administration of histamine effects a kind of desensitization at least comparable in results to that achieved by more laborious and more costly specific desensitization procedures and that it is often of greater duration than that obtained by the latter.

Successful relief of allergic disorders by histamine as reported lends support to the theory that histamine or a histamine-like substance is produced in the body as an intermediate step in the body's reaction to the majority of, if not all, allergens. Its production may be illustrated schematically as follows:

Allergen → Sensitive body cells → Histamine (or histamine-like "H-substance") → Sensitive body tissue (vascular system) → Allergic reaction

Thus, on the basis of this hypothesis of the genesis of allergic reactions, when histamine is given according to a desensitization schedule, the sensitive body tissue, which formerly responded with a so-called allergic reaction to histamine produced by the impact of the allergenic substance on sensitive body cells, may be accustomed to histamine (desensitized) in such a way that it no longer reacts to the stimulus of allergen-produced histamine. The chain of events leading to the allergic manifestation is interrupted one step farther along than is the case with specific desensitization therapy where the response of sensitive body cells to the allergen itself is inhibited.

Obviously, the use of nonspecific histamine desensitization treatment has definite advantages which should appeal to both doctor and patient alike, since this method offers the doctor an immediate therapeutic means of relieving his patient, and also, in many cases, of obtaining a lasting remission, at a time when a search for specific irritants is exceedingly difficult. Of considerable importance is the fact that nonspecific desensitization therapy with histamine is considerably more economical than most specific desensitization procedures.

The side reactions reported are not uncommon to desensitization methods, and, while they illustrate the necessity for careful dosage and adequate supervision of the patient, they should not militate against the use of histamine any more than similar side effects deter the use of specific desensitization measures.

SUMMARY

Histamine dihydrochloride was employed parenterally in very small doses, increased from 0.00001 mg. to 0.1 mg. according to a definite desensitization schedule, in a series of 53 cases of urticarial-like allergic skin manifestations. Forty patients were completely relieved, 11 were improved, and only 2 were not benefited. Remission of symptoms obtained by histamine treatment has lasted for twelve to twenty-two months.

Twenty-eight other patients with various allergic syndromes were given histamine similarly, with a favorable response in the majority of instances.

These disorders, included infantile and senile eczema, rhinitis, pruritus ani, pruritus vulvae, atrophic arthritis, and edematous conditions.

Reactions of varying severity were experienced by some patients to injections of histamine. Reduction of the dosage in most cases effected control of the reaction and permitted effective continuation of therapy. However, careful administration of histamine is essential; its use is apparently contraindicated in patients with certain cardiac disorders.

CONCLUSIONS

Complete remission of symptoms may be obtained in many cases of urticaria by the injection of minute doses of histamine dihydrochloride, increased regularly by small amounts in accordance with a desensitization schedule.

The similar use of histamine may be beneficial in other allergic skin conditions, particularly those evidenced by pruritus, edema, and exudation.

A word of caution is indicated with regard to indiscriminate histamine therapy.

II. TREATMENT OF GASTRODUODENAL ULCERATION

The etiology of peptic ulcer is not yet definitely known, and various theories have been promulgated to account for its occurrence. Some of these hypotheses hold that an allergic reaction is at the etiologic base of many cases of gastroduodenal ulceration. The possible mechanism of such a reaction, involving liberation of a histamine-like substance and an ensuing vascular response which may eventuate in peptic ulcer formation, has been discussed in Part I.

Since desensitization with histamine was effective in correcting allergic manifestations such as urticaria and eczema, it was decided to employ a similar desensitization schedule in certain cases of peptic ulcer, on the assumption that peptic ulcer is caused by the influence of some allergen or allergens. This decision was aided by the fact that several patients treated for cutaneous allergic disorders simultaneously experienced relief of ulcer symptoms.

Eleven cases of peptic ulcer syndromes, in several of which the history suggested that allergy played a causative role, were given histamine injections, using the 1:1,000 solution of histamine dihydrochloride. The same schedule as for urticaria was followed—0.00001 mg. intradermally increasing to 0.1 mg. in successive subcutaneous doses at forty-eight-hour intervals. A twelfth patient with gastric carcinoma was also given histamine for its favorable effect on pain, appetite, etc. The brief case reports which follow illustrate the response of these 12 patients to histamine therapy.

CASE REPORTS

CASE 1.—L. D. E., male, aged 12 years, was sent in for an allergy examination to determine the cause of rhinitis. He had had an appendectomy two years previously, asthma the summer before, and head cold and sinusitis for the past few weeks. There was a history of allergy in the family and hay fever in the father. During examination the patient complained of pain in abdomen with marked tenderness over epigastrium. X-ray examination revealed characteristic ulcer deformity. Belladonna administration and a modified Sippy diet were begun. On the first day of his hospital stay an urticarial rash was noted over the abdomen, which itched intensely. A dose of 0.00001 mg. of histamine was given intradermally for the urticaria, and both urticaria and epigastric pain were relieved within twenty-four hours; previously it had been necessary to administer $\frac{1}{8}$ gr. of morphine for

relief. Pain returned the third day, and histamine was again given, with astonishing relief. The patient was kept in the hospital for ten days on a modified Sippy diet and belladonna, but also received histamine according to the desensitization schedule every other day, with no recurrence of pain or other discomfort. After a six weeks' diet therapy x-ray showed an apparently normal intestine. There has been no recurrence within eighteen months.

Attention is called to the relief of urticaria and pain in this case.

CASE 2.—Negro, male, aged 50 years or more, a day laborer, complained of pain characteristic of duodenal ulcer. X-ray examination showed definite deformity of the cap; this deformity was not influenced by atropine. Sippy diet, belladonna, and sedatives were given for two weeks; the patient remained in bed for one week. This treatment did not bring about relief of pain and discomfort. Since it was evident that his condition was not improving and since it was difficult to keep him on a diet, surgery was considered. First, however, in view of the good results in Case 1, 0.0001 mg. of histamine was injected intradermally. The patient noticed almost immediately that his sensation of fullness was diminished, and he felt more comfortable and remained so for forty-eight hours; then symptoms returned. Subsequent small doses of histamine, increased every other day for approximately 20 doses to 0.1 mg., brought about apparently complete relief. Twelve months later, working twelve hours daily at hard manual labor and thinking very little about diet, this man is symptom-free.

Attention is called to the fact that the patient continued to experience fullness, soreness, acute pain, and distress until after histamine therapy was begun. From that point on there was a distinct diminution in his symptoms, with relative comfort after the fourth injection.

CASE 3.—J. L. P., male, aged 60 years, a railroad employee, had black tarry stools, extreme weakness, general gastric upset, and distress for one week before consulting me. I had attended him for perforations in the duodenum ten years previously, at which time an operation was performed which involved the simple closure of two ulcers with uneventful recovery. The patient was put to bed under treatment with an oral proprietary preparation, belladonna, and modified Sippy diet for one week. After stools had cleared, diet therapy was continued. X-ray examination showed constant deformity of ulcer, slight narrowing of pylorus, and retention after six hours. Medical treatment was continued, but as this did not give patient appreciable relief, gastroenterostomy was advised and declined. Mr. P. was convinced that he had a malignancy and would not listen to any suggestion of surgery, despite discomfort. He was finally persuaded to try histamine therapy, keeping a daily record of his sensations and symptoms following each dose. The following are excerpts from the record:

"5/11/38 At one-thirty P.M., after having taken a shot at eleven A.M., am feeling fairly comfortable in my stomach.

"5/13 Had no unpleasant feeling in my stomach after lunch today. Lay down for a couple of hours, got up feeling better than for a couple of weeks.

"5/15 Did not wake with pain last night as usual.

"5/17 Had real severe pain last night but ate a canned pear which I think caused the trouble.

"5/19 I am feeling lots better after each shot now, only I am growing a bit weak.

"After the fourth shot the only change I notice is that I am feeling better each day. . . . I did not keep further daily data as the only effects I felt were that I was improving daily. I would have turned this in sooner but was waiting to see what the results would be and to see if the shots had made a complete cure, which I am convinced they have, as I have had absolutely no pain since the fourth shot. My appetite is good, and I eat practically anything I want, but I try not to eat too much. Have lost only one day from work since the thirteenth of August and this is the twenty-eighth of September."

These results indicate the evident relief with histamine, without observation of dietary restrictions. Several weeks later, however, x-ray examination indi-

cated a defect which, I believe, may be the result of adhesions following previous perforation, since the patient is symptom-free.

CASE 4.—B. A., white male, aged 37 years, complained of duodenal ulcer. He stated that he felt exactly as he did prior to his last perforation—he had been operated upon twice previously with simple closure of perforations. Physical examination was essentially negative except for ulcerlike tenderness and marked undernourishment. There was a history of two attacks of asthma in 1934, with occasional difficulty in breathing and nasal obstruction. Since the patient required considerable building up for operation and since there was no indication of pyloric obstruction, he was put on an oral proprietary preparation, belladonna, and a modified Sippy diet, with occasional sedatives. After three weeks he did not improve subjectively and histamine therapy was initiated, beginning with 0.00001 mg. intradermally. There was a noticeable improvement in comfort during the next forty-eight hours. Histamine injections were continued, with relief of symptoms, and the patient was discharged some three weeks later, apparently well and symptom-free. After a short interval, during which time no injections were given, symptoms returned, and a posterior gastroenterostomy was performed. The stomach was normal and the duodenum showed two healed, old ulcers, but no evidence of induration of recent ulcer. Adhesions in the upper gastrointestinal tract were corrected. Following the operation recovery was uneventful except for hicough which responded to treatment, but convalescence was delayed by the patient's mental state. He would not take nourishment, complained of every trifle, and, in general, was very hard to manage. Since such notable improvement had resulted from histamine prior to operation, it was again employed in an attempt to aid in recovery. Injections of small doses brought about a decided change for the better: the patient's appetite returned, his attention to personal appearance improved, and he wanted to sit up and get out of bed. Saline injections were given for one week as a control procedure and symptoms returned, to be relieved by further histamine therapy.

In this case the preoperative use of histamine enabled us to build up the patient sufficiently to stand operation, and its postoperative use was of benefit in his recovery. Gastroenterostomy described above was performed in September, 1938; in April, 1939, the patient complained of soreness and distress in epigastrium. He was again given 13 injections of histamine, following which his symptoms subsided and he gained 6 pounds. The patient is apparently well at present.

CASE 5.—H. D., male, aged 40 years, complained of pain in stomach, gas, vomiting, blood in stools, and fullness after meals. Physical examination revealed an undernourished male, with tenderness in epigastrium. X-ray examination showed normal esophagus and stomach; a large crater on posterior wall of the duodenal bulb, a wide inflammatory ring about ulcer, and a diverticulum on the descending portion of duodenum. The patient was given 0.00001 mg. of histamine and on the following day felt better than he had for three weeks previous. A bland diet was given without alkali neutralization or other medication. After two injections of histamine the patient was discharged from the hospital under the care of his physician who continued with a regular schedule for histamine injections. He improved clinically from the first injection. Two months after histamine therapy was begun, x-ray examination showed a fluoroscopically normal stomach, with no hypersecretion; moderate constant deformity of duodenal bulb, but no visible niche; no retention at six hours, but moderate degree of hypermotility. His physician reports clinical relief has been maintained to date.

CASE 6.—E. McK., female, aged 41 years, had pain in epigastrium and distress characteristic of ulcer. She had been treated for peptic ulcer since October, 1938, at which time she had a hemorrhage. Two years previously a gastroenterostomy had been done. Physical examination was essentially negative except for tenderness in epigastrium to left of old laparotomy scar. X-ray examination on admission revealed evidence of a stomal ulcer with

a well-functioning gastroenterostomy. After a blood transfusion the patient was put on histamine therapy and a bland diet, with apparent clinical improvement. She was given injections at the hospital for ten days and then discharged; she received subsequent injections at the office. She was apparently symptom-free one month after beginning histamine therapy, and although she had slight retention at six hours, the roentgenologist reported that no stomal ulcer could be demonstrated. After a remission of several weeks, the patient again complained of distress in epigastrium. X-ray examination check showed a well-functioning gastroenterostomy; a small niche was observable at the stoma. Barium did not pass through the duodenum, there being a 10 per cent retention at six hours; the remainder of the barium passed normally into the cecum. The patient was advised to re-enter the hospital for observation, but a bed was unavailable at the time and she has since failed to appear for treatment.

In this case definite clinical improvement was noted, particularly subjective improvement, regardless of the x-ray findings. The patient was almost deaf, and it was exceedingly difficult to obtain information from her; also her mental state was not the most favorable for this type of cooperative study.

CASE 7.—C. B. A., female, aged 48 years, complained of pain in the right upper quadrant and back, with hematemesis, indigestion, fullness in epigastrium, headache, sinusitis, nervousness, and hot flushes. General examination revealed only tenderness over the gall bladder on deep pressure; symptoms and history were suggestive of gall bladder disease or peptic ulcer. X-ray examination showed good gall-bladder shadows, with no evidence of calculi; in gastrointestinal series the stomach appeared normal, with normal peristalsis and well-filled duodenal bulb. There was a minimal deformity of the duodenal cap, without definite niche. The patient had a chronic duodenal ulcer and was put on a bland diet without antacid medication and allowed to be ambulant. A daily record of experiences following histamine injections was kept. After the sixth dose she began to feel comfortable and did not spit up further blood. She received from 0.00001 mg. to 0.1 mg. of histamine in 17 injections.

I was unable to again x-ray this patient after the period of histamine injections, so no definite conclusion can be made of the degree of healing of her ulcer which may have taken place. Following the sixth injection, however, she was clinically free of symptoms for an indefinite period of time, slight symptoms recurring after twelve months.

CASE 8.—F. L. E., male, aged 31 years, a painter, had pain in his right side, nagging in character, with nausea, occasional vomiting, constipation, and nervous indigestion for six years. Physical examination was essentially negative except for poorly kept teeth and pyorrhea. X-ray examination after barium enema showed no evidence of pathology. In gastrointestinal series the chest and esophagus were fluoroscopically normal, there were considerable hypersecretion in stomach, spastic pylorus, marked deformity of duodenal bulb, and definite niche; the second portion of the duodenum was redundant. There was 20 per cent retention in stomach at six hours, but progress of the barium was normal beyond the stomach. The patient was put on a bland diet, and injections of histamine were begun. Immediately following the first dose (within three to four hours), he experienced definite relief of sensation of fullness, gas, and pain in the side; his appetite improved, and he felt generally better and stronger. Despite a complete series of histamine injections he still continued to experience some discomfort in his side. A confirmatory x-ray examination was made; it showed no abnormality in esophagus or stomach, but revealed a large ulcer pocket just distal to the pyloric sphincter. Retention in the stomach with considerable spasm about the pylorus was also noted.

In this case a combination of aluminum hydroxide, diet, rest, and histamine would probably have effected a more definite response. Subjectively, this

patient was the only one of those with known ulcer who did not respond satisfactorily to histamine alone. However, both x-ray and gastric analysis indicated improvement.

CASE 9.—J. M. G., male, aged 41 years, has since 1929 suffered from gastrointestinal symptoms suggestive of duodenal ulcer. He has taken soda, mineral oil, etc., for distention, gas, and discomfort most of his life. An x-ray examination in 1936 showed that he had an ulcer. A second x-ray examination in 1937, by a different laboratory, did not demonstrate an ulcer. He was given 14 injections of histamine (0.00001 mg. to 0.01 mg.) at intervals of every other day; this therapy resulted in remission of symptoms for seven months, after which time he returned for further prophylactic injections, stating that for this period he had been symptom-free.

CASE 10.—O. G. C., male, aged 46 years, was x-rayed in 1937 and told he had an ulcerated stomach. An x-ray examination in 1939 revealed a normal stomach and upper intestinal tract, but definite colitis and symptoms of duodenal ulcer. Because his teeth and gums were poor, his teeth were removed. Histamine injections were given before and during the removal of teeth with lasting abatement of symptoms. The discomfort was relieved immediately and has not returned to date.

CASE 11.—W. I. G., male, aged 40 years, was previously x-rayed, but no definite evidence of ulcer was found. He presented a history of indigestion, gas distention, nausea, gastric upsets, constipation, relief from soda, etc. A series of histamine injections afforded him remission of symptoms for several months. At present, after a nine months' symptom-free period, he is again experiencing some discomfort.

CASE 12.—J. W., aged 39 years, was admitted to service on Aug. 23, 1939, with diagnosis of gastric ulcer. He was discharged and re-admitted on Sept. 20, 1939. He gave a history of syphilitic infection fifteen years previously with requisite treatment. X-ray examination revealed advanced infiltration of carcinoma in cardia and lower esophagus. The patient was, therefore, not a proper subject for histamine therapy, but histamine was given in an attempt to improve his appetite, and to determine what effect, if any, it might have on his feeling of well being. A noticeable improvement in comfort, in appetite, and in mental outlook resulted. The patient became hungry and had no difficulty with his food. His general condition was also improved considerably prior to his departure to a clinic for surgical attention.

DISCUSSION

It may be very significant from the standpoint of etiology of peptic ulcer that the same dosage schedule of histamine injections employed successfully in treating urticaria and other skin lesions is effective in affording remission of ulcer symptoms. An allergic reaction has been proved to underlie the urticarial disorders described in Part I, and reasoning by analogy one may assume that cases of peptic ulcer responsive to histamine are similarly caused and perpetuated.

Of course, it is not justifiable to assume on the basis of results in this small initial series that all cases of peptic ulcer have an etiologic background of allergy; in fact, the effectiveness of histamine may conceivably be that of the substance per se, rather than of its desensitizing influence against external or internal irritants. However, there is sufficient evidence of improvement of ulcer symptoms in individuals of an allergic diathesis after injections of histamine to warrant the assertion that allergy plays a certain causative role. This assumption derives further support from the observation of simultaneous relief of allergic skin disorders in patients whose peptic ulcer symptoms are also benefited.

Psychic factors should be considered to a certain extent in evaluating the efficacy of this type of therapy. However, that they probably play a minor role is evident from the finding that in at least one case, symptoms which had been relieved by histamine injections returned when saline solution was substituted for the histamine; when histamine therapy was reinstituted, the symptoms once more disappeared.

The small doses of histamine injected did not stimulate gastric acid secretion, as demonstrated by gastric analyses. This finding is of importance in view of the fact that peptic ulcer is invariably aggravated by increased acid flow. An effective therapeutic method should have no stimulating influence on acid production, and histamine therapy in the dosage employed apparently satisfies this criterion.

SUMMARY

Eight patients with peptic ulcer syndromes and demonstrable ulcers were treated with a bland diet, supplemented by injections of histamine according to a desensitization schedule, without any attempt at acid neutralization. Similar treatment was given in three other patients with similar symptom syndromes but without demonstrable evidence of ulcer. A twelfth patient with advanced carcinoma of the esophagus and stomach was also given a series of histamine injections during his hospitalization period.

Subjectively, the ulcer patients experienced relief of symptoms, in most instances following the fourth histamine injection, and they were able to enjoy a fuller diet than the routine in similar cases. There was a return of a sensation of well-being in the person with cancer, with increase in appetite as well as in comfort. Remission of symptoms in duodenal ulcer syndromes without demonstrable lesions was apparently dependent on the total number of injections and the amount of histamine given.

Studies of gastric function did not demonstrate hypersecretion of acid from small doses of histamine; rather, there appeared to be some inhibition of secretory activity, and in one case actual lowering of the acid curve.

X-ray studies of gastric functional activity and the regression of previously demonstrated niches in several cases confirmed subjective evidence of improvement or healing of ulcer lesions.

No marked reactions to injection of histamine, but occasional headache and generalized aching, with a feeling of weakness, were experienced by some patients.

CONCLUSIONS

It would appear from the results obtained and the observations made in this small series of cases that repeated injections of small amounts of histamine, in increasing doses, can well be used to augment the more recognized treatment of peptic ulcer, supplementing diet, rest, and acid neutralization.

It would also appear that the use of histamine desensitization therapy in patients with ulcer symptom syndromes, but without demonstrable ulcers, can afford these patients a remission of symptoms.

The favorable response of patients with peptic ulcer syndromes to histamine in small increasing doses would indicate an allergic basis for the develop-

ment or maintenance of the syndrome in these cases. The response is similar, in respect to the type of therapy involved, to that observed in cases of urticaria and other skin lesions of a proved allergic etiology.

From a consideration of the various factors involved, it would appear that the use of histamine in peptic ulcer has a certain place, particularly in those patients who are of the so-called allergic diathesis type and manifest other symptoms of an allergic character.

Further investigation of the histamine therapy of peptic ulcer is recommended.

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REFERENCES

1. Editorial note: Antihistamine Therapy and Treatment of Asthma, *J. A. M. A.* 100: 250, 1933.
2. Auld: Treatment of Asthma, *Brit. M. J.* 1: 171, 1928.
3. Bray: Recent Advances in Allergy, ed. 3, Philadelphia, 1937, P. Blakiston's Son & Co.
4. Dzsinich: Histamine in Treatment of Allergic Conditions, *Klin. Wehnschr.* 14: 1612, 1935; Abstr., *J. A. M. A.* 105: 2191, 1935.
5. Ernstene and Banks: Histamine Therapy of Pruritus; Preliminary Report, *J. A. M. A.* 100: 328, 1933.
6. Fiessinger and Gajdos: Reactogenotherapy of Recedivating Urticaria Ionization, *Presse méd.* 43: 1913, 1935.
7. Friedlaender and Petow: Migraine as Symptom of Biliary Disease; Treatment With Histamine, *Med. Klin.* 23: 1498, 1927.
8. Gajdos: Reactogen Therapy by Histamine Ionization in Recurrent Urticaria, *Rev. gén. de clin. et de thérap.* 48: 517, 1934.
9. Hansel: Allergy of the Nose and Paranasal Sinuses, St. Louis, 1936, The C. V. Mosby Co.
10. Israel, Jacob, and Meyer: Persistent Urticaria Treated by Cutaneous Scarification With Histamine, *Strasbourg méd.* 96: 43, 1936.
11. Meineri: Morphine, Histamine, and Peptone in Therapy of Lupus, *Dermosiflografo* 8: 185, 1931.
12. Stahl and Masson: Histamine; Cutaneous Application in Asthmaticiform Dyspnea, *Compt. rend. Soc. de biol.* 111: 1003, 1933.
13. Thiberge: Histamine Therapy of Asthma, New Orleans *M. & S. J.* 85: 39, 1932.
14. Wise and Sulzberger: Yearbook of Syphilis and Dermatology, 1936.
15. Tuft: Clinical Allergy, Philadelphia, 1937, W. B. Saunders Co.
16. Saylor and Wright: Studies on Two Cases of Urticaria From Cold Sensitivity and of the Effect of Histamine Treatment, *Am. J. M. Sc.* 192: 388, 1936.
17. Collens, Lerner, and Fialka: Insulin Allergy; Treatment With Histamine, *Am. J. M. Sc.* 188: 528, 1934.
18. Kling and Saslin: Histamine Iontophoresis in Rheumatic Conditions, etc., *Arch. Phys. Therapy* 18: 333, 1937.
19. Bloomfield and Pollands: Studies of Gastric Secretion, *J. A. M. A.* 92: 1508, 1929.
20. Bartosch: Histamine in Anaphylaxis in the Guinea Pig, *Klin. Wehnschr.* 14: 307, 1935.
21. Dzsinich and Paul: Treatment of Allergic Disorders, *Klin. Wehnschr.* 15: 1754, 1936.
22. Wangenstein and Loucks: Studies in Intestinal Obstruction; Absorption of Histamine, *Arch. Surg.* 16: 1089, 1928.
23. Essex, Markowitz, and Mann: Physiologic Action of Bee Venom, *J. Applied Physiol.* 94: 209, 1930.
24. Benson and Semenov: Allergy in Relation to Bee Stings, *J. Allergy* 1: 105, 1930.
25. Mueller: Periodic Edema of the Hand With a Seven-Day Cycle; Treatment With Histamine, *M. Clin. North America* 13: 1543, 1930.
26. Bray: A Case of Physical Allergy; Localized and Generalized Allergic Type of Reaction to Cold, *J. Allergy* 3: 367, 1932.
27. Horton: Hypersensitiveness to Cold, *Proc. Staff Meet., Mayo Clin.* 9: 477, 1934.
28. Eusterman and Balfour: The Stomach and Duodenum, Philadelphia, 1936, W. B. Saunders Co.

CLINICAL CHEMISTRY

THE ACID-BASE BALANCE IN THE PLASMA AND BLOOD CELLS OF NORMAL NONPREGNANT, PREGNANT, AND PUERPERAL WOMEN*

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THE acid-base equilibrium in the plasma of normal pregnant women has been intensively studied by many investigators, but there is still some disagreement concerning the extent and significance of the variations involved. Oard and Peters,¹ Muntwyler, Limbach, Bill, and Myers,² Kydd,³ Kydd, Oard, and Peters,⁴ Myers, Muntwyler, and Bill,⁵ and Nee, Mull, Muntwyler, and Myers⁶ have recently reviewed the literature and have made excellent contributions toward the solution of this complex problem. It is generally agreed that during gestation there is a reduction in the carbon dioxide content and in the protein percentage of the plasma, which is associated with a comparable diminution in the total base, but there is still some difference of opinion as to whether these changes affect the hydrogen-ion concentration. The present study was designed to investigate the plasma and whole blood and by calculation to determine alterations in the blood cells.

MATERIAL

Complete studies were made on (a) ten normal nonpregnant women of the childbearing age (nurses and ambulatory patients with minor afflictions that ordinarily would not disturb the acid-base or the water balance), (b) ten primigravidas and ten multigravidas who were in the third trimester of pregnancy and who showed no signs or symptoms of disease, and (c) ten normal postpartum women who were tested seven to nine days after delivery. All individuals received the regular (mixed) hospital diet.

METHODS AND CALCULATIONS

A portion of the blood was drawn under anaerobic conditions into tubes containing dry sodium oxalate and oil and was analyzed for carbon dioxide content and capacity, oxygen content and capacity, chlorides, phosphates, cell volume, and water. A second portion, similarly treated, was centrifuged under oil, and the supernatant plasma was used for determining the carbon dioxide content and capacity, hydrogen-ion concentration, total protein, nonprotein nitrogen, chlorides, phosphates, and water. The final portion was collected in a clean dry tube and allowed to clot, the serum being used for analyses of sodium, calcium, magnesium, and sulfates. Potassium was determined on the solid

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residues obtained when whole blood and plasma were dried to constant weight in the water content determinations, as previously described.⁷

The concentrations of the various blood cell constituents were calculated from determined data on the concentrations in whole blood and plasma and on the cell volumes, using the generalized formula:

$$\text{Concentration in the whole blood} = \frac{\text{Concentration in the plasma} \times \text{plasma volume} + \frac{X \times \text{cell volume}}{100}}{100}, \text{ where } X \text{ is the concentration in the cells.}$$

The various plasma and cell constituents are reported in terms of milliequivalents.* The total acid represents the summation of the anions bound with the basic elements. Total acid equals $[\text{proteinate}]^- + [\text{HCO}_3]^- + [\text{Cl}]^- + [(\text{HPO}_4)^{-}] + (\text{H}_2\text{PO}_4)^{-}] + [\text{SO}_4]^{-}$. On the other hand, the total base equals $[\text{Na}]^+ + [\text{K}]^+ + [\text{Ca}]^{++} + [\text{Mg}]^{++}$, which represents the sum of the cations.

Cell volumes were determined by using Plass and Rourke sedimentation tubes† and rotating them at 3,000 r.p.m. for thirty minutes.

Oxygen and carbon dioxide contents and capacities were determined by the manometric method of Van Slyke and Neill,⁸ using the gas apparatus and technique of Van Slyke.⁹ Factors of 0.1 and 0.5 volume per cent were subtracted from the oxygen content and capacity, respectively, for the dissolved and the free oxygen. If we assume that one molecule of oxygen combines with one molecule of hemoglobin, the corrected oxygen capacity divided by 2.24 gives the millimols of hemoglobin.

The hydrogen-ion concentration of the plasma was determined by a K-type potentiometer with a quinhydrone microelectrode similar to that described by Cullen.¹⁰

Volumes per cent of carbon dioxide were converted into milliequivalents present as bicarbonate by the following equation^{11, 12}:

$$[\text{BHCO}_3] = [\text{CO}_2] - \frac{[\text{CO}_2]}{10^{\text{pH} - \text{pK}^1} + 1}$$

in which $[\text{CO}_2]$ represents the millimolar concentration of total CO_2 or $\frac{\text{Volume per cent } \text{CO}_2 \times 0.01976}{0.044}$. pK^1 values of 6.10 and 6.13 were used for

plasma and whole blood, respectively. The factor $\frac{[\text{CO}_2]}{10^{\text{pH} - 6.10} + 1}$ represents the concentration of free carbonic acid in the plasma.

The alkali reserve was calculated from the volume per cent carbon-dioxide combining power by means of the formula used to determine the amount of BHCO_3 .

Carbon dioxide tension was calculated from the carbonic acid concentration in the plasma by the following formula:

$$p - \text{CO}_2 \text{ mm.} = \frac{[\text{H}_2\text{CO}_3]_s}{0.0327}$$

*A milliequivalent, or millimol (mEq. or mM), of a given constituent represents $\frac{1}{1000}$ part of its hydrogen equivalent or of its molecular weight, respectively, per kilogram of water. For example, 1.0 mEq. of sodium equals $\frac{1}{1000}$ of its atomic weight divided by 1.008 (its hydrogen equivalent), or $\frac{23}{1,000} \div 1.008 = 0.023$.

†Manufactured by Macalaster, Bicknell Co., 40 Wendell St., Cambridge, Mass.

The Folin-Wu method¹³ was used to determine the nonprotein nitrogen in plasma and whole blood.

The total nitrogen of plasma was determined by the micro-Kjeldahl method.¹⁴ After the nonprotein nitrogen was subtracted, the protein nitrogen was multiplied by the factor 6.25, to obtain the total protein percentage, which was converted into milliequivalents of base combined with protein, [BP], by the formula of Peters, Wakeman, Eisenman, and Lee,¹⁵ $[BP] = 1.072 P$ ($pH - 5.04$), in which an albumin-globulin ratio of 1.8 was assumed.

The Wilson and Ball procedure for chlorides in whole blood and plasma was employed.¹⁶ The milligrams per cent of sodium chloride divided by 5.85 represent the milliequivalents of chlorides in 1,000 c.e.

Inorganic phosphorus was determined by the method of Youngburg and Youngburg.¹⁷ The milligrams per cent of phosphorus was reduced to milliequivalents of $[HPO_4]^{--} + [H_2PO_4]^-$ per liter by the factor $\frac{18}{31.04}$, accepting Henderson's¹⁸ estimate of the proportion of primary and secondary phosphates.

In the sulfate determinations the benzidine sulfate was prepared according to Wakefield,¹⁹ but instead of proceeding to a colorimetric comparison, a titrimetric method was employed, using 0.02 normal sodium hydroxide with phenol red as the indicator. Assuming that each atom of sulfur combines with two equivalents of monovalent base, 1 mg. per cent of sulfate is equivalent to 0.208 mEq. of combined base.

The specific gravities were determined by weighing 2.0 c.e. of plasma or whole blood in a 25 c.e. silica crucible. The water content was obtained by drying the specimen at 105° C. to constant weight.

Potassium was determined on the dried material remaining in the crucible, which was treated with one or two drops of concentrated sulfuric acid and ashed overnight at a low heat in an electric muffle. The ash was dissolved in a few drops of dilute hydrochloric acid and water. The solution obtained from the plasma specimen was transferred to a 15 c.e. centrifuge tube and evaporated to dryness, while that from the whole blood was transferred to a 15 c.e. graduated centrifuge tube, made up to 10 c.e. with water, and centrifuged to separate the insoluble iron oxide; an aliquot portion of the clear supernatant solution was transferred to another centrifuge tube and evaporated to dryness. The residues from the serum and whole blood were analyzed for potassium by the method of Shohl and Bennett.²⁰ One milligram per cent of potassium is equivalent to $\frac{1}{3.91}$, or 0.256 mEq. per liter.

The serum sodium was determined by the zinc uranyl acetate technique of Salit.²¹ The direct method previously described by one of us²² was used to determine the cell sodium, the concentration values of which are recorded.

One milligram per cent of sodium is equivalent to $\frac{1}{23}$, or 0.0434 mEq. per liter.

Calcium was determined by the Clark and Collip²³ modification of the Kramer-Tisdall procedure.²⁴ One milligram per cent of calcium is equivalent to $2 \times \frac{1}{4.0}$, or 0.5 mEq. per liter.

TABLE I
ACID-BASE EQUILIBRIUM IN PLASMA OF NORMAL NONPREGNANT, LATE PREGNANT, AND PUERPERAL WOMEN
(Concentrations of constituents are reported in terms of milliequivalents per kilogram of water unless otherwise stated.)

MATERIAL	NONPREGNANT		LATE PREGNANT PRIMIGRAVIDAS		LATE PREGNANT MULTIGRAVIDAS		POST PARTUM (7-9 DAYS)	
	AVERAGE	RANGE	AVERAGE	RANGE	AVERAGE	RANGE	AVERAGE	RANGE
No. of cases	10		10		10		10	
pH	7.39	7.33-7.47	7.37	7.30-7.48	7.40	7.33-7.48	7.39	7.32-7.43
[Cl] ⁻	111.3	106.5-115.9	111.3	103.1-114.5	113.2	104.9-121.8	112.0	107.3-117.0
[HCO ₃] ⁻	27.4	24.3-30.9	24.9	20.3-27.8	24.2	21.6-27.3	28.1	26.4-29.4
[Protenate] ⁻	18.4	15.6-21.7	15.9	13.5-20.1	15.6	13.6-20.3	18.4	16.5-21.1
[HPO ₄] ⁻ + [H ₂ PO ₄] ⁻	2.5	2.3-2.8	2.4	2.1-2.7	2.3	2.0-2.7	2.5	2.2-2.9
[SO ₄] ⁻	0.5	0.2-1.0	0.6	0.3-1.2	0.5	0.2-1.2	0.7	0.2-1.3
Total acids	160.1	153.5-168.5	155.1	147.4-161.2	155.8	148.6-162.8	161.7	155.4-166.4
[Na] ⁺	156.7	151.1-164.7	151.9	150.7-157.5	151.3	140.8-162.7	158.0	151.2-162.6
[K] ⁺	4.8	4.2-6.0	5.0	4.1-6.0	5.2	4.1-6.1	4.7	4.2-5.3
[Ca] ⁺⁺	5.5	4.6-6.4	5.2	4.8-6.0	5.1	4.7-5.9	5.8	4.6-6.4
[Mg] ⁺⁺	2.4	2.0-2.8	2.3	1.9-2.9	2.4	1.9-2.9	2.3	1.9-3.2
Total bases	169.2	161.0-176.7	164.2	161.6-169.1	164.0	152.9-176.0	170.8	162.9-176.6
p-CO ₂ mm.	43.7	32.1-55.5	41.9	31.2-50.6	37.3	31.7-44.7	45.2	41.1-54.0
H ₂ CO ₃ mM	1.43	1.05-1.81	1.37	1.02-1.65	1.22	1.04-1.46	1.48	1.34-1.76
N.P.N. mg. per cent	30.3	25.5-36.5	31.6	25.0-40.4	31.9	25.0-44.4	38.1	28.2-44.2
Alkali reserve	31.5		28.7		27.7		30.6	

TABLE II
ACID-BASE EQUILIBRIUM IN CELLS OF NORMAL NONPREGNANT, LATE PREGNANT, AND PUERPERAL WOMEN
(Concentrations of constituents are reported in terms of milliequivalents per kilogram of water unless otherwise stated.)

MATERIAL	NONPREGNANT		LATE PREGNANT MULTIGRAVIDAS		LATE PREGNANT MULTIGRAVIDAS		POST PARTUM (7-9 DAYS)	
	AVERAGE	RANGE	AVERAGE	RANGE	AVERAGE	RANGE	AVERAGE	RANGE
[Cl] ⁻	79.2	72.4 - 86.9	74.9	59.2 - 87.8	71.7	45.2 - 87.1	72.6	57.8 - 85.0
[HCO ₃] ⁻	24.0	18.7 - 26.6	20.7	16.0 - 23.9	19.5	14.1 - 22.1	23.1	20.2 - 25.7
[Protein] ⁻	45.3		45.5		49.2		48.4	
[HPO ₄] ⁻ + [H ₂ PO ₄] ⁻	3.2	2.6 - 4.5	3.2	2.1 - 4.5	3.3	2.0 - 5.9	3.6	2.9 - 4.8
Total acids	151.7		144.5		143.7		147.7	
[K] ⁺	136.2	127.2 - 150.6	138.8	129.2 - 149.1	139.7	122.0 - 164.3	146.9	131.1 - 150.2
[Na] ⁺			16.6		16.6			
O ₂ capacity volume per cent	16.79		15.95		15.35		18.94	
O ₂ content volume per cent	7.62		7.73		8.22		9.71	
pH estimated	7.17		7.15		7.18		7.17	
Alkali reserve	24.4		26.2		25.1		23.0	

The method of Denis²⁵ was employed for the determination of magnesium. One milligram per cent of magnesium is equivalent to $2 \times \frac{1}{2.43}$, or 0.819 mEq. per liter.

The base bound by hemoglobin, or base protein, $[\text{BP}]_c$, was calculated according to the equation of Van Slyke, Wu, and McLean:²⁶

$$[\text{BP}]_c = 3.35 [\text{Hb}]_c (\text{pH}_c - 6.74) + [\text{O}_2]_c (0.25 \text{ pH}_c - 1.18)$$

where $[\text{Hb}]_c$ represents millimols of hemoglobin per kilogram of water; $[\text{O}_2]_c$, the millimols of oxygen per kilogram of water, and pH_c , the hydrogen-ion concentration within the cells as estimated from a graph using plasma pH data.²⁵ The values thus obtained may not represent the base actually bound by the hemoglobin in human blood, since the equation was derived from data on horse hemoglobin, but they undoubtedly have some value for comparative purposes.

RESULTS

The average values for the various constituents (Table I), except those for the pH, are in accord with the reports of previous investigators.¹⁻⁶ The average pH value is the same in the pregnant women as in the nonpregnant, thus confirming the observations of Kydd, Oard, and Peters,⁴ and others. The total acid and the total base are diminished approximately 5.5 mEq. during late pregnancy but return to the nonpregnant level within seven to nine days after delivery. Reductions of the bicarbonate and of the proteinate ions account chiefly for the decrease in total acid, while the lowered sodium explains the reduced total base, which is associated with a lowered alkali reserve. The average values for carbon dioxide tension and for carbonic acid, as calculated from the total carbon dioxide content of the plasma, are slightly reduced in late pregnancy but return to normal shortly after delivery.

Since all values recorded for the blood cells (Table II) have been calculated from the analyses of plasma or serum and of whole blood, and from hematocrit determinations, it is obvious that they are subject to undue variations as the result of a possible summation of analytic errors. Average values for cell chloride and bicarbonate in the nonpregnant group agree with those reported by Hastings, Sendroy, McIntosh, and Van Slyke,¹² but they are lower in the groups of women in late pregnancy; this confirms the observations of Muntwyler, Myers, and Way.²⁸ The cell proteinates and phosphates show no significant variations in the different groups. During pregnancy the total acids in the cells are approximately 7.6 mEq. per liter below the nonpregnant level, and by the seventh to the ninth days post partum they have only partially returned to normal, being still 4.0 mEq. under the nonpregnant average.

The cell potassium, which represents most of the total base in the cells, is slightly elevated in the pregnant groups, the rise being associated with an increased hemoglobin content per kilogram of water, as previously reported.¹ During the early puerperium the cell potassium rises still higher, and there is no indication as to when it returns to normal. Values for the sodium content of the cells are available only for the pregnant groups, and consequently no comparison can be attempted. The alkali reserve is increased slightly in the

cells during gestation but is decreased in the plasma. After delivery both serum potassium and plasma alkali reserve return promptly to the normal non-pregnant level, in spite of the persistent elevation of the cell potassium.

DISCUSSION

The recorded pregnancy changes in the various plasma constituents confirm the observations of previous investigators and require no comment.

During pregnancy the diffusible constituents of the blood cells vary generally with the alterations in the plasma to maintain a Donnan type of equilibrium. The total base of the cells is not influenced by the reduction in the total base of the plasma but actually rises slightly because it consists largely of non-diffusible potassium, which is closely associated with the equally fixed hemoglobin. This situation probably explains the increased alkali reserve of the cells. The amount of base bound with hemoglobin to form a proteinate is governed by the pH and the carbon dioxide content of the blood. In general, the variations in the acid-base balance of the cells are due directly to alterations in the concentrations of the diffusible constituents of the plasma.

An interpretation of the acid-base balance in pregnant and puerperal women has been presented in a previous communication dealing with pulmonary ventilation.²⁹

SUMMARY

1. A detailed study of the acid-base equilibrium of the plasma and cells in normal ten nonpregnant, twenty pregnant, and ten puerperal women is reported.
2. The reduction of the total acid constituents of the plasma, which occurs during pregnancy, is balanced by a corresponding reduction in the total base, with both factors returning to normal early in the puerperium.
3. The pH of the plasma remains at the normal nonpregnant level during pregnancy and early puerperium.
4. The free carbonic acid and the carbon dioxide tension of the plasma fall slightly in late pregnancy but return to the nonpregnant level shortly after delivery.
5. The total acid constituents in the cells are slightly diminished in late pregnancy but return to normal within nine days after delivery.
6. The potassium concentration in the cells, which is increased in late pregnancy, shows a further rise during the early puerperium.
7. The alkali reserve of the cells is increased during pregnancy but falls to the nonpregnant level within a few days after confinement.

REFERENCES

1. Oard, H. C., and Peters, J. P.: The Concentration of Acid and Base in the Serum in Normal Pregnancy, *J. Biol. Chem.* **81**: 9, 1929.
2. Muntwyler, E., Limbach, N., Bill, A. H., and Myers, V. C.: Acid-Base Equilibrium of Blood in Pathological Conditions; Changes Observed in Toxemias of Pregnancy, *J. Biol. Chem.* **90**: 607, 1931.
3. Kydd, D. M.: Hydrogen-Ion Concentration and Acid-Base Equilibrium in Normal Pregnancy, *J. Biol. Chem.* **91**: 63, 1931.

4. Kydd, D. M., Oard, H. C., and Peters, J. P.: The Acid-Base Equilibrium in Abnormal Pregnancy, *J. Biol. Chem.* 98: 241, 1932.
5. Myers, V. C., Muntwyler, E., and Bill, A. H.: The Acid-Base Balance Disturbance of Pregnancy, *J. Biol. Chem.* 98: 253, 1932.
6. Nice, M., Mull, J. W., Muntwyler, E., and Myers, V. C.: The Acid-Base Balance of the Blood During Normal Pregnancy and Puerperium, *Am. J. Obst. & Gynec.* 32: 375, 1936.
7. Oberst, F. W., and Plass, E. D.: Water Concentration of the Blood During Pregnancy, Labor, and the Puerperium, *Am. J. Obst. & Gynec.* 31: 61, 1936.
8. Van Slyke, D. D., and Neill, J. M.: Determination of Gases in Blood and Other Solutions by Vacuum Extraction and Manometric Measurement, *J. Biol. Chem.* 61: 523, 1924.
9. Van Slyke, D. D.: Note on a Portable Form of the Manometric Gas Apparatus and on Certain Points in the Technique of Its Use, *J. Biol. Chem.* 73: 121, 1927.
10. Cullen, G. E.: Improved Form of Quinhydrone Electrode, *J. Biol. Chem.* 83: 535, 1929.
11. Austin, J. H., Cullen, G. E., Hastings, A. B., McLean, F. C., Peters, J. P., and Van Slyke, D. D.: Studies of Gas and Electrolyte Equilibria in Blood. I. Technique for Collection and Analysis of Blood, and for Its Saturation With Gas Mixtures of Known Concentration, *J. Biol. Chem.* 54: 121, 1922.
12. Hastings, A. B., Sendroy, J., Jr., McIntosh, J. F., and Van Slyke, D. D.: Studies of Gas and Electrolyte Equilibria. XIII. The Distribution of Chloride and Bicarbonate in the Blood of Normal and Pathological Human Subjects, *J. Biol. Chem.* 79: 193, 1928.
13. Folin, O., and Wu, H.: A System of Blood Analysis, *J. Biol. Chem.* 38: 81, 1919.
14. Hawk, P. B., and Bergeim, O.: *Practical Physiological Chemistry*, ed. 10, Philadelphia, 1931, P. Blakiston's Son & Co., p. 449.
15. Peters, J. P., Wakeman, A. M., Eisenman, A. J., and Lee, C.: Total Acid-Base Equilibrium of Plasma in Health and Disease. X. The Acidosis of Nephritis, *J. Clin. Investigation* 6: 517, 1929.
16. Wilson, D. W., and Ball, E. G.: A Study of the Estimation of Chloride in Blood and Serum, *J. Biol. Chem.* 79: 221, 1928.
17. Youngburg, G. E., and Youngburg, M. V.: Phosphorus Metabolism. I. A System of Blood Phosphorus Analysis, *J. LAB. & CLIN. MED.* 16: 158, 1930.
18. Henderson, L. J.: The Theory of Neutrality Regulation in the Animal Organism, *Am. J. Physiol.* 21: 427, 1908.
19. Wakefield, E. G.: Colorimetric Determination of Total Inorganic Sulfates in Blood Serum, Urine, and Other Body Fluids, *J. Biol. Chem.* 81: 713, 1929.
20. Shohl, A. T., and Bennett, H. B.: A Micro Method for the Determination of Potassium as Iodoplatinate, *J. Biol. Chem.* 78: 643, 1928.
21. Salit, P. W.: A New Triple Acetate Method for Sodium Determinations in Biological Materials, *J. Biol. Chem.* 96: 659, 1932.
22. Oberst, F. W.: The Determination of Sodium in Human Red Blood Cells, *J. Biol. Chem.* 108: 153, 1935.
23. Clark, E. P., and Collip, J. B.: A Study of the Tisdall Method for the Determination of Blood Serum Calcium, With a Suggested Modification, *J. Biol. Chem.* 63: 461, 1925.
24. Kramer, B., and Tisdall, F. F.: A Simple Technique for the Determination of Calcium and Magnesium in Small Amounts of Serum, *J. Biol. Chem.* 47: 475, 1921.
25. Denis, W.: The Determination of Magnesium in Blood, Plasma, and Serum, *J. Biol. Chem.* 52: 411, 1922.
26. Van Slyke, D. D., Wu, H., and McLean, F. C.: Studies of Gas and Electrolyte Equilibria in Blood; Factors Controlling Electrolyte and Water Distribution in Blood, *J. Biol. Chem.* 56: 765, 1923.
27. Taylor, H.: The Hydrogen-Ion Concentration of Blood Corpuscles, *J. Physiol.* 63: 343, 1927.
28. Muntwyler, E., Myers, V. C., and Way, C. T.: The Distribution of Chloride and Bicarbonate Between Plasma and Cells in the Blood of Various Pathological Conditions, *J. Biol. Chem.* 92: 721, 1931.
29. Plass, E. D., and Oberst, F. W.: Respiration and Pulmonary Ventilation in Normal Nonpregnant, Pregnant, and Puerperal Women, *Am. J. Obst. & Gynec.* 35: 441, 1933.

THE MAGNESIUM CONTENT OF THE BLOOD SERUM AND URINE*

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BECAUSE of the increasing importance of magnesium in the field of cardiovascular-renal disease and because of the relative dearth of data in the literature, the present investigation of the magnesium content of the blood serum and urine was undertaken.

The patients studied comprised 201 consecutive medical ward cases, divided almost equally between both sexes. A total of 325 blood serum determinations was performed. Insofar as could be determined the patients had no disturbance of mineral metabolism. In general, a fasting blood magnesium test was made although it has been shown that no noticeable difference exists in the magnesium content of the blood before and after meals.¹ The method employed for the determination of magnesium was the modification of the Titan yellow dye method recently perfected by Haury,² a method so essentially simple and dependable that it ought to mark a great step forward in the clinical investigation of magnesium metabolism.

The statement is seen repeatedly in the literature that the amount of blood magnesium is remarkably constant, varies within very narrow limits only, and is practically uninfluenced by pathologic processes.^{3, 4} Yet a careful perusal of the literature disclosed very marked divergences in the recorded data.

The patients were divided into a noncardiac group with a wide miscellany of diseases, a cardiac group, and a nephritic group. The combined cardiac and noncardiac group totaled 197 patients in whom 312 blood serum magnesium tests were performed. The average magnesium value was 2.1 mg. per cent which agreed fairly well with the results of Beecher⁵ (1.8 to 2.3), Bomskov⁶ (1.7 to 2.6), Velluz and Velluz⁷ (2.00) (1.62 to 2.40), Brookfield⁸ (2.04) (1.89 to 2.19), Cope⁹ (2.06) (1.82 to 2.63), and Walker and Walker¹⁰ (1.5 to 2.9), but was considerably below the values obtained by Greenberg and others¹¹ (2.74) (2.0 to 3.6), Briggs¹² (2.23 to 2.50), Wacker and Fabrig¹³ (2.4) (2.03 to 2.97), Denis¹⁴ (1.6 to 3.5), Cohen¹⁵ (2.56) (2.09 to 2.74), Marriott and Howland¹⁶ (2.2 to 3.5), Kramer and Tisdall¹⁷ (2.3 to 4.0), Watchorn and McCance¹⁸ (2.3 to 2.65), Watchorn¹⁹ (2.2 to 2.6), and McCance and Watchorn²⁰ (2.00 to 4.05). A noteworthy feature is the marked individual variations we found (1.23 to 3.54) in support of the findings of Greenberg and co-workers.¹¹ Evidence of the serum magnesium does not vary within such narrow limits as was hitherto supposed.

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NONCARDIAC GROUP

The noncardiac group included 94 patients in whom 138 magnesium tests were performed. The average serum magnesium was 2.12, with extremes of 1.37 to 3.11. A division of this group into smaller groups of patients with definite diseases (such as pneumonia, irritable colon, cholelithiasis, bronchial asthma) disclosed no great variations from the general average. The pregnancy group (comprising 4 cases) tended to show somewhat lower values in support of the findings of Watchorn and McCance.¹⁸ The miscellaneous section comprised a great variety of diseases, including erysipelas, Hodgkin's disease, scleroderma, Henoch's purpura, bronchiectasis, psoriasis, thromboangiitis obliterans, ulcerative colitis, and chronic lymphatic leucemia.

TABLE I
BLOOD SERUM MAGNESIUM VALUES

DIAGNOSIS	NO. OF PATIENTS	NO. OF TESTS	AVG. BLOOD SERUM (MG. %)	BLOOD SERUM (MG. %) (EXTREMES)
<i>A. Noncardiac Group</i>				
Pneumonia	12	16	1.96	1.23-2.56
Cholelithiasis	9	17	2.02	1.38-2.60
Irritable colon	8	11	2.14	1.69-2.83
Pyelitis and nephroptosis	6	8	1.96	1.58-2.40
Bronchial asthma	5	10	2.12	1.66-2.91
Diabetes mellitus	4	7	2.06	1.62-2.58
Pregnancy	4	4	1.99	1.68-2.30
Duodenal ulcer	3	4	2.28	2.04-2.44
Miscellaneous	43	61	2.21	1.37-3.11
Noncardiac total	94	138	2.12	1.37-3.11
<i>B. Cardiac Group</i>				
Ambulatory hypertensives	23	34	2.25	1.67-3.31
Ward hypertensives	31	57	2.27	1.63-3.41
Arteriosclerotic heart disease	35	66	2.23	1.57-3.54
Miscellaneous heart disease	14	17	2.30	1.29-3.29
Cardiac total	103	174	2.25	1.29-3.54
Total groups A and B	197	312	2.19	1.23-3.54
<i>C. Chronic Nephritis</i>				
Chronic nephritis	4	13	3.31	1.59-4.60

CARDIAC GROUP

The cardiac group numbered 103, of whom 54 had hypertensive heart disease. The magnesium level in this condition is still a mooted question. Wacker and Fahrig¹³ state that magnesium is the only mineral found in increased concentration in the serum in essential hypertension. Their average magnesium value was 2.41 as compared with their normal of 2.28. In a group of 15 hypertensive patients without severe renal damage Walker and Walker¹⁰ found an average value of 2.36, as compared with their normal value of 2.2 in normal active young adults, and 2.3 in miscellaneous medical and surgical hospital cases. In 1923 Weil, Guillaumin, and Weismann-Netter²¹ found an increased serum magnesium in a few cases of essential hypertension, but to quote Walker and Walker:¹⁰ "These figures deviate so far, however, from the standards used by other authors that comparison is not possible." In our series were 23 ambulatory

hypertensive patients whose magnesium levels were 2.5 (1.67 to 3.31) and 31 ward hypertensive patients of whom many were decompensated with values of 2.27 (1.63 to 3.41). The grand average for the hypertensive group was 2.26, as compared with our normals of 2.12. The increase is very slight but is in conformity with the results obtained by the authors quoted above. Whether any significance is to be attached to so small a difference is certainly open to question.

The arteriosclerotic heart disease group numbered 35 patients in whom 66 magnesium determinations were effected. The average value was 2.23 mg. per cent, with extremes of 1.57 to 3.54. In the miscellaneous heart disease group were 14 patients in whom 17 blood magnesium studies were made. The group was heterogenous and included 4 cases of chronic rheumatic heart disease, 2 of congenital heart disease, one of acute rheumatic fever, 3 of subacute bacterial endocarditis, one of syphilitic heart disease, one of scarlatinal heart block,³¹ and 2 of thyrotoxic heart disease. The average magnesium value for the foregoing group was 2.24 (1.29 to 3.30). It is noteworthy that the magnesium value for nonhypertensive patients with cardiac disorders is elevated to practically the same degree as in patients in the hypertensive group, though again emphasis must be laid on the fact that the increase is very slight (Table I).

NEPHRITIC GROUP

The question of the magnesium content of the serum in renal insufficiency is still in a state of flux. One group affirms that there is no retention of magnesium in renal insufficiency. In this group are Denis and Hobson,²² who obtained figures of 2.2 to 2.9 in 19 patients, Denis,²³ working in experimental nephritis, and Fishberg.²⁴ In 1923 Salvesen and Linder²⁵ reported 15 patients in whom they sometimes found a reduction and sometimes an increase in magnesium (1.5 to 4.6). Somewhat similar results were obtained a year later by Rabinowitch²⁶ (1.6 to 3.1). That magnesium is retained in renal insufficiency has been asserted by another group of investigators. Included are Becher,⁵ Becher and Hamann,²⁷ who found values of 2.7 to 5.7 in 10 cases; Walker and Walker,¹⁰ who found abnormally high serum magnesium values in 5 hypertensive persons with renal damage (2.07 to 4.20); Cope,⁹ who in 1936 found the serum magnesium considerably elevated in 5 patients with renal damage increased after intensive alkali administration (4 patients with readings over 4.00 and one with a reading of 6.84); Hirschfelder,^{28, 29} who in 1934 reported serum magnesium figures often considerably raised in renal inadequacy; and Brookfield,⁸ who confirmed the increase of serum magnesium in renal dysfunction in a series of 21 cases (values did not exceed 3.93).

In the present investigation, 4 cases of nephritis were studied. The average reading was 3.31 (1.89 to 4.60).

CASE 1.—L. F., a female, aged 24 years, had had chronic nephritis of a mild character for several years but no renal insufficiency. Blood magnesium values taken several weeks apart were 1.89 and 2.55.

CASE 2.—R. C., a male, aged 63 years, had hypertension with definite chronic glomerulonephritis but only slight renal insufficiency. Blood magnesium determinations within a period of two weeks were 2.90, 2.86, and 2.06 keeping pace with the patient's clinical improvement.

CASE 3.—C. B., a female, aged 17 years, had advanced chronic glomerulonephritis. Blood magnesium was 4.36 on admission, 4.17 on the sixth day, 3.99 on the seventh, and 4.36 on the thirteenth. She received 1 ounce of magnesium sulfate by mouth on the sixth and seventh days, and 1.5 ounces on the ninth, tenth, eleventh, and thirteenth days, as well as an intramuscular injection of 2 c.c. of 50 per cent magnesium sulfate daily from the eighth to thirteenth day inclusive.

CASE 4.—M. J., a hypertensive negress, aged 38 years, had chronic glomerulonephritis with extreme degree of renal insufficiency. Initial blood magnesium on admission to the hospital (July 16, 1937) was 2.8. She received intramuscular injections of 2 c.c. of 50 per cent magnesium sulfate twice daily from August 3 to August 16 inclusive. Blood magnesium level on August 5 was 2.90, on August 12, 3.68, and on August 16, 4.60. The patient became comatose on August 19 and died the following day. Post-mortem examination confirmed the presence of an advanced glomerulonephritis. The results obtained bear out Brookfield's findings that an increase of serum magnesium is almost invariably present in renal dysfunction and corroborate his failure to disclose magnesium levels sufficient to produce coma as advanced by Hirschfelder.²⁹

URINARY MAGNESIUM

There are very few recorded experimental studies covering the level of the urinary magnesium. Cope⁹ recorded the basic urinary output of magnesium in one patient as 50 mg. per day. Walker and Walker¹⁰ found the twenty-four-hour urinary output in a group of normal active young adults to average 103 mg. (32.5 to 307.0 mg.) and 86 mg. (5 to 243 mg.) in a group of miscellaneous medical and surgical hospital patients. In 8 young adults Weber³⁰ recently reported the average daily urinary excretion of magnesium to be 83.9 to 132.1 mg. In our series the average daily excretion of 57 patients on admission to the hospital was found to be 105.5 mg., with values ranging from 17.3 to 285.0 mg. The values agree closely with those obtained by Walker and Walker. Such high variations as we obtained might readily be expected a priori when the great range of the individual diet is considered.

In several patients intramuscular injections of magnesium sulfate were given with a consequent reflection in the urinary output. In a hypertensive patient the initial urinary output was 45.3 mg. After 2 c.c. of 50 per cent magnesium sulfate were injected intramuscularly twice daily for four days, the urinary output rose to 102.9 and to 140.7 mg. the subsequent day.

In a patient with arteriosclerotic heart disease the initial urinary value was 80.0, rose to 158 the day following the intravenous injection of 15 c.c. of 10 per cent magnesium sulfate, reached 298.0 following the injection of 2 c.c. of 50 per cent magnesium sulfate intramuscularly twice daily for four days, and rose to a peak of 416.1 after the intramuscular injections were continued for eleven days more. No correlation could be established between the urine output and the blood level.

CONCLUSIONS

1. A total of 325 determinations of the blood serum magnesium were made in 201 medical cases with no disturbance of mineral metabolism.
2. A total of 312 determinations in 197 of the patients (excluding the 4 nephritic patients in the series) showed an average value of 2.19 mg. per cent (1.23 to 3.54).
 - (a) A total of 138 magnesium tests in 94 noncardiac patients showed an average value of 2.12 per cent (1.37 to 3.11).

(b) Ninety-one magnesium tests in 54 hypertensive persons gave an average value of 2.26 (1.63 to 3.41).

(c) Sixty-six magnesium determinations in 35 arteriosclerotic heart disease patients showed an average value of 2.23 (1.57 to 3.54).

(d) Seventeen magnesium tests in 14 patients with various other forms of heart disease showed an average magnesium value of 2.24 (1.29 to 3.30).

3. In 4 cases of chronic nephritis with varying degrees of renal insufficiency the blood serum magnesium level was elevated to almost double the normal value (3.31) (1.89 to 4.60).

4. The average twenty-four-hour urinary output of magnesium in 57 persons was 105.5 mg. (17.3 to 285.0).

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REFERENCES

1. Watchorn, E.: Thesis, Cambridge, 1929.
2. Haury, V. G.: Modification of Titan Yellow Method for Determination of Small Amounts of Magnesium in Biological Fluids, *J. LAB. & CLIN. MED.* 23: 1079, 1938.
3. Sholl, A. T.: Mineral Metabolism—Calcium and Magnesium, *Ann. Rev. Biochem.* 2: 207, 1933.
4. Peters, J. P., and Van Slyke, D. D.: Quantitative Clinical Chemistry, Baltimore, 1932, Williams & Wilkins Co., Vol. 1.
5. Becher, E.: Der Magnesiumgehalt des Blutes unter normalen und pathologischen Verhältnissen, insbesondere bei Nierenkrankheiten, *Klin. Wchnschr.* 11: 202, 1932.
6. Bomskov, C.: Determination of Magnesium in Blood With 8-hydroxy quinoline, *J. Biol. Chem.* 99: 17, 1932.
7. Velluz, L., and Velluz, J.: Sur les variations physiologiques du magnésium sériques, *Compt. rend. Soc. de biol.* 117: 417, 1934.
8. Brookfield, R. W.: Magnesium Content of Serum in Renal Insufficiency, *Quart. J. Med.* 6: 87, 1937.
9. Cope, C. L.: Base Changes in the Alkalosis Produced by the Treatment of Gastric Ulcer With Alkalies, *Clin. Sc.* 2: 287, 1936.
10. Walker, B. S., and Walker, E. W.: Normal Magnesium Metabolism and Its Significant Disturbances, *J. LAB. & CLIN. MED.* 21: 713, 1936.
11. Greenberg, D. M., Lucin, S. P., Mackey, M. A., and Tufts, E. V.: Magnesium Content of the Plasma and Red Corpuscles in Human Blood, *J. Biol. Chem.* 100: 139, 1933.
12. Briggs, A. P.: A Colorimetric Method for the Determination of Small Amount of Magnesium, *J. Biol. Chem.* 52: 349, 1922.
13. Wacker, L., and Fahrig, C.: Über die mineralischen und lipoiden Bestandteile des Blutserums bei der essentiellen Hypertension un Vergleich zu den physiologischen Verhältnissen, *Klin. Wchnschr.* 11: 762, 1932.
14. Denis, W.: Determination of Magnesium in Blood, *J. Biol. Chem.* 41: 363, 1920.
15. Cohen, H.: Magnesium Content of the Cerebrospinal and Other Body Fluids, *Quart. J. Med.* 20: 173, 1927.
16. Marriott, W. McK., and Howland, J.: A Micro-method for the Determination of Calcium and Magnesium in Blood Serum, *J. Biol. Chem.* 32: 233, 1917.
17. Kramer, B., and Tisdall, F. T.: The Distribution of Sodium, Potassium, Calcium and Magnesium Between the Corpuscles and Serum of Human Blood, *J. Biol. Chem.* 53: 241, 1922.
18. Watchorn, E., and McCance, R. A.: Inorganic Constituents of Cerebrospinal Fluid. II. The Ultrafiltration of Calcium and Magnesium From Human Sera, *Biochem. J.* 26: 54, 1932.
19. Watchorn, E.: Calcium and Magnesium in Some Pathological Sera, *Quart. J. Med.* 18: 288, 1925.
20. McCance, R. A., and Watchorn, E.: Inorganic Constituents of Cerebrospinal Fluid. I. Calcium and Magnesium, *Quart. J. Med.* 24: 371, 1931.
21. Weil, M., Guillaumin, C., and Weismann-Netter, R.: Le calcium et le magnésium sériques chez l'adulte normal, hypertendu ou atheromateux, *Compt. rend. Soc. de biol.* 88: 732, 1923.
22. Denis, W., and Hobson, S.: A Study of the Inorganic Constituents of the Blood Serum in Nephritis, *J. Biol. Chem.* 55: 183, 1923.
23. Denis, W.: Study of the Inorganic Constituents of the Blood in Experimental Nephritis, *J. Biol. Chem.* 56: 473, 1923.

24. Fishberg, A. M.: *Hypertension and Nephritis*, ed. 3, Philadelphia, 1934, Lea and Febiger, p. 29.
25. Salvesen, H. A., and Linder, G. C.: Observations on the Inorganic Bases and Phosphates in Relation to the Problem of Blood and Other Body Fluids in Bright's Disease and in Heart Failure, *J. Biol. Chem.* 58: 617, 1923.
26. Rabinowitch, I. M.: On the Relative Proportions of Sodium, Potassium, Calcium and Magnesium in Blood Plasma in Renal Disease, *J. Biol. Chem.* 52: 667, 1925.
27. Becher, E., and Hamann, K.: Studien über das Verhalten des Magnesium im Organismus insbesondere in Blut, *Deutsches Arch. f. klin. Med.* 173: 560, 1932.
28. Hirschfelder, H. D.: Effect of Renal Insufficiency Upon Plasma Magnesium and Magnesium Excretion After Ingestion of Magnesium Sulfate, *J. Biol. Chem.* 104: 647, 1934.
29. Hirschfelder, H. D.: Clinical Manifestations of High and Low Plasma Magnesium Dangers of Epsom Salt Purgation in Nephritis, *J. A. M. A.* 102: 1138, 1934.
30. Weber, R. J.: Excretion of Some Purgative Salts of Magnesium, *Proc. Soc. Exper. Biol. & Med.* 37: 55, 1937.
31. Bernstein, Mitchell: Auriculoventricular Dissociation Following Scarlet Fever, *Am. Heart J.* 16: 582, 1938.

INFLUENCE OF GLYCEROL ON GLYCEMIA IN NORMAL AND DIABETIC INDIVIDUALS*

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THE relationship of substances other than hexoses, especially those having three carbon atoms, to carbohydrate metabolism has received considerable attention. Our knowledge of the metabolism of some of these substances is, however, in many respects fragmentary. In this paper we shall concern ourselves with the metabolism of glycerol. Although much work has been done on this subject, there are certain important phases which have received only scant attention. Experimental work tending to elucidate these problems will be reported.

REVIEW OF LITERATURE

The metabolism of glycerol may be considered from the following standpoint: (1) glycogenesis, (2) antiketogenesis and ketolysis, (3) influence of hypoglycemic shock, (4) influence on blood sugar.

1. *Glycogenesis.* Catron and Lewis¹ found that the amounts of glycogen formed in the liver after two- and three-hour periods of absorption of glycogen from the gastrointestinal tracts of white rats were equal to those observed after absorption of equivalent amounts of glucose over the same periods. Shapiro² has completely confirmed these observations. Chambers and Deuel³ observe that a practically complete conversion of glycerol into glucose was exhibited by two phlorhizinized dogs. Sansum and Woodratt⁴ found that d,l-glycerol aldehyde was quantitatively converted into glucose as determined by measuring glycosuria in deglycogenated phlorhizinized dogs. They argued that this precludes the possibility of glycerol having a greater utilization rate than glucose itself in diabetic persons.

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2. Antiketogenesis and ketolysis. Although the antiketogenic action of glycerol was first noted by Hirschfeld in 1895, it was most convincingly demonstrated by Satta's experiments.^{2*} Shapiro² found that equivalent amounts of glucose and glycerol had equal ketolytic properties.

3. Influence on hypoglycemic shock. Voegtlin and Dunn⁵ observed that the symptoms of insulin shock (coma and convulsions) were completely relieved by intravenous or intraperitoneal injections of glycerol. Noble and Macleod,⁶ on the contrary, found that glycerol did not affect the symptoms of insulin shock in the slightest degree.

4. Influence on respiratory quotient. The only work on this subject is by McCann and Hannon,⁷ who compared the respiratory quotients in three normal persons after the ingestion of glycerol and glucose. They found that whereas a marked rise in respiratory quotient was produced by glucose, no rise occurred with glycerol. It should be mentioned that glucose and glycerol are not strictly comparable, for the respiratory quotient of glucose is 1, whereas that of glycerol is approximately 0.86.

5. Influence on concentration of blood sugar. Voegtlin, Thompson, and Dunn⁸ found that glycerol given to fasted rabbits produced a hyperglycemia of considerable extent and duration. This increase was not due to an increase in concentration of the blood. Studies on the influence of glycerol on glycemia in man have been scant. Kneip⁹ observed that glycerol given in doses of 0.5 to 0.6 Gm. per kilogram of body weight did not influence the blood sugar of one diabetic and four normal children. Belrens¹⁰ also found that a dose of 30 Gm. of glycerol did not give rise to hyperglycemia in one diabetic and three normal children.

Fats, such as tristearin or triolein, when completely saponified yield approximately ten parts by weight of glycerol to ninety parts by weight of higher fatty acids. As previously mentioned, Sansum and Woodyatt contend that glycerol does not have a greater utilization rate than glucose itself in diabetic persons. It has been customary in estimating the glucose value of a diabetic diet to consider fat as yielding 10 per cent by weight of glucose, and to put that glucose in the same category as that derived from starch.

Investigations on the influence of glycerol on blood sugar have been fragmentary and inadequate. In this paper results of experiments will be reported on the influence of glycerol on blood sugar on a fairly large number of normal and diabetic individuals. The question of the utilization rate of glycerol in diabetic persons will to some extent, for reasons given below, be elucidated by the results obtained.

METHOD OF PROCEDURE

Experiments were performed on 13 normal and 10 diabetic persons. Eighty grams of glycerol,* in sufficient aqueous solution to constitute a total of 500 c.c., were given to each person in the morning after a fast of fourteen hours. Specimens of blood were taken from the normal individuals before, and forty-five minutes, two, and three hours after the ingestion of the glycerol. The sugar

*In the recovery of glucose from glycerol, two molecules of glycerol yield one molecule of glucose and four atoms of hydrogen.
 $2C_3H_8O_2 \rightarrow C_6H_{12}O_6 + 4H$, or 80 Gm. of glycerol yield 78.2 Gm. of glucose.

TABLE I

CONCENTRATION OF DEXTROSE IN THE BLOOD OF NORMAL INDIVIDUALS AT THE FASTING LEVEL
AND AFTER THE INGESTION OF 80 GM. OF GLYCEROL

CASE NO.	BLOOD SUGAR			
	FASTING LEVEL	FORTY-FIVE MINUTES POSTCIBAL	TWO HOURS POSTCIBAL	THREE HOURS POSTCIBAL
1	106	100	98	
2	86	95	111	
3	97	102	115	
4	103	97	147	117
5	94	104	128	104
6	97	134	126	119
7	95	105	116	118
8	87	80	87	84
9	100	90	111	90
10	92		109	105
11	100	130	147	125
12	98	103	103	98
13	110	112	108	
Means	97	104	116	106

TABLE II

CONCENTRATION OF DEXTROSE IN THE BLOOD OF DIABETIC PERSONS AT THE FASTING LEVEL
AND AFTER THE INGESTION OF 80 GM. OF GLYCEROL

CASE NO.	BLOOD SUGAR			
	FASTING LEVEL	ONE HOUR POSTCIBAL	TWO HOURS POSTCIBAL	THREE HOURS POSTCIBAL
1	138	141	145	144
2	171	169	192	198
3	182	225	308	286
4	125	138	135	133
5	166	185	194	173
6	246	282	333	318
7	200	246	254	282
8	194	215	217	210
9	230	286	351	355
10	361	366	400	411
Means	201	225	253	254

TABLE III

CONCENTRATION OF DEXTROSE IN THE BLOOD OF DIABETIC PERSONS AT THE FASTING LEVEL
AND AFTER THE INGESTION OF 75 GM. OF GLUCOSE

CASE NO.	BLOOD SUGAR		
	FASTING LEVEL	FORTY-FIVE MINUTES POSTCIBAL	TWO HOURS POSTCIBAL
1	242	400	428
2	250	328	364
3	150	250	300
4	124	364	422
5	176	256	231
6	168	300	378
7	234	356	
8	238	410	374
9	222	378	468
10	98	224	264
11	224	322	364
Means	193	327	359

content of the blood was determined by the Folin-Wu method. In the diabetic patients the blood specimens were taken at the same time with one exception, namely, one hour instead of forty-five minutes after ingestion.

ANALYSIS AND DISCUSSION OF RESULTS

As mentioned in the review of the literature, there has been a paucity of study on the influence of glycerol on glycemia. Voegtlin, Thompson, and Dunn⁸ found that glycerol given to fasted rabbits produces a hyperglycemia of considerable extent and duration. Kneip⁹ and Belhrens,¹⁰ on the other hand, observed that glycerol had no influence on the blood sugar in several normal and diabetic children.

Table I shows the concentrations of the blood sugar in 13 normal individuals at the fasting level and after the ingestion of 80 Gm. of glycerol. A definite rise in the concentration of the blood sugar is noted in 8 of the 13 persons.

Table II shows the concentration of glucose in the blood of 10 diabetic persons at the fasting level and after the ingestion of 80 Gm. of glycerol. In 8 of the 10 cases a definite rise in the concentration of the blood sugar is observed. This rise may be collated with that of a comparable group of diabetic persons who received 75 Gm. of glucose (Table III). It will be noted that the rise in the concentration of the blood sugar when glucose is given is markedly greater than after glycerol.

The question arises: To what extent is this difference the result of a difference in the rates of absorption of the two substances from the human gastrointestinal tract? There has, unfortunately, been no experimental work on this subject. As already mentioned, Catron and Lewis¹ found that the amounts of glycogen formed in the liver after two- and three-hour periods of absorption of glycerol from the gastrointestinal tract of white rats were equal to those observed after the absorption of equivalent amounts of glucose over the same periods. This would indirectly indicate that no significant difference in the rates of absorption of glucose and glycerol from the gastrointestinal tract exists.

Another question is whether the rise in the concentration of blood sugar after glycerol is produced by an increase in the concentration of the blood. As previously noted, Voegtlin, Thompson, and Dunn⁸ did not observe any increase in the concentration of the blood in rabbits after the ingestion of glycerol.

The point to be considered now is the capacity of the diabetic person to utilize glycerol as compared with glucose. As noted above, Sansum and Woodyatt⁴ contend that glycerol does not have a greater utilization rate than glucose in diabetic persons.

The greatest glycemia, and consequently glycosuria, occurs in diabetic patients during the two- to three-hour period immediately following a meal. This degree of glycemia varies even when different hexoses are given. Thus galactose will give rise to a lower blood sugar curve than an equal amount of glucose. Consequently, the diabetic person can utilize galactose better than glucose. This is strikingly more so with glycerol. As already shown, the blood sugar curve when glycerol is ingested is markedly lower than when an equivalent amount of glucose is taken. It must be concluded then that the diabetic individual has a greater capacity to utilize glycerol than glucose.

It should be mentioned that this matter is of more 'scientific than practical importance, for the amount of glycerol derived by the saponification of fat is small. Furthermore, the glycerol is produced at a slow rate.

CONCLUSIONS

1. The ingestion of glycerol produces an increase in the concentration of blood sugar in a majority of normal and diabetic individuals.
2. Diabetic persons have a greater capacity to utilize glycerol than glucose.

REFERENCES

1. Catron, L. F., and Lewis, H. B.: Glycogen Formation From Glycerol, *J. Biol. Chem.* 84: 553, 1929.
2. Shapiro, I.: The Comparative Glycogenic and Ketolytic Action of Glucose and Some Carbohydrate Intermediates, *J. Biol. Chem.* 108: 373, 1935.
- 2a. Quoted by Shapiro, *Ibid.*
3. Chambers, W. H., and Deuel, H. J.: Metabolism of Glycerol in Phlorhizin Diabetes, *J. Biol. Chem.* 65: 21, 1925.
4. Sansum, W. C., and Woodyatt, R. T.: The Behavior of dl-Glyceric Aldehyde in the Normal and Diabetic Organism, *J. Biol. Chem.* 24: 327, 1916.
5. Voegtlin, C., and Dunn, E. R.: Antagonistic Action of Glycerol on Insulin Intoxication, *J. Pharmacol. & Exper. Therap.* 25: 168, 1925.
6. Noble, E. C., and Macleod, J. J. R.: The Influence of Sugars and Other Substances on the Toxic Effects of Insulin, *Am. J. Physiol.* 64: 547, 1923.
7. McCann, W. S., and Hannon, R. R.: Respiratory Exchange Following the Ingestion of Glucose, Glycerol, etc., *Bull. Johns Hopkins Hosp.* 34: 73, 1923.
8. Voegtlin, C., Thompson, J. W., and Dunn, E. R.: Hyperglycemia Produced by Glycerol, *J. Biol. Chem.* 64: 639, 1935.
9. Kneip, J.: Blutzuckerbeeinflussung durch perorale Fett- bzw. Glycerinzufuhr bei Kindern, *Monatschr. f. Kinderh.* 64: 359, 1936.
10. Behrens, H. O.: Ueber den Einfluss des Glycerin auf den Blutzuckerspiegel, *Monatschr. f. Kinderh.* 63: 368, 1935.

GUANIDINE-LIKE SUBSTANCES IN BLOOD*

VII. GUANIDINE AND OTHER BLOOD CONSTITUENTS IN EXPERIMENTAL ANOXEMIA

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IT HAS been pointed out in the previous paper of this series¹ that a tendency for hyperguanidinemia has been reported in a number of conditions exhibiting spasticity or convulsions. Although a part of these abnormal findings or possibly all in some diseases, may be ascribed to faulty analytical methods or faulty interpretation, there is still sufficient data to make additional study worth while in this direction.

As already pointed out¹ recent work seems to have entirely disproved the thesis of a hyperguanidinemia in parathyroid insufficiency.²⁻⁸ The high values observed by previous investigators seem to be entirely due to faulty methods of

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possibly to blood concentration from dehydration. There is more evidence, however, that the convulsive states associated with the toxemias of pregnancy and related disorders may have a definite relationship to the concentration of guanidine in the blood. Malmejae and co-workers,⁹⁻¹¹ Minot and Cutler,¹² and Andes, Andes, and Myers¹³ have found a definite increase in the blood guanidine concentration in the toxemias of pregnancy, especially in true eclampsia. Traut and McFate¹⁴ observed hyperguanidinemia in a case of dysmenorrhea accompanied by convulsions. The entirely negative findings of de Wesselow and Griffith,¹⁵ Stander,¹⁶ and Krieger¹⁷ throw considerable doubt on the value of these findings. Andes and co-workers¹³ have pointed out that hyperguanidinemia in eclampsia is usually accompanied by some degree of renal insufficiency, and suggest this factor as the probable cause of most of the increase (renal insufficiency is usually, if not invariably, accompanied by an increase in the blood guanidine concentration¹⁸). They did note, however, a definite hyperguanidinemia in two cases of eclampsia with convulsions not accompanied by nitrogen retention, and furthermore, pointed out that patients in convulsions tended to show higher values. They also demonstrated a definite increase in the blood guanidine in *normal* women after delivery.

Ellis, Neal, and Frazier¹⁹ have reported an increased blood guanidine content in epileptic persons, the increase roughly paralleling the severity of the clinical symptoms. Although their work has not been confirmed, the changes observed are so marked as to apparently relate the blood guanidine change to the convulsant state. In addition, Dodd and Minot^{20, 21} and also Schumacher,²² have noted a hyperguanidinemia in the convulsive states of childhood. It is the opinion of some that dehydration is an important factor in producing the blood changes in this last set of conditions, but such has not been proved to be the entire cause.

In an attempt to elucidate the entire problem, Andes and Emerson¹ administered various convulsive drugs to rabbits and dogs, but obtained entirely negative results as far as blood guanidine was concerned. There still remained the possibility that anoxemia of the muscles and other tissues might be an important factor. Dordi's observation²³ of the presence of a hyperguanidinemia in pneumonia is some evidence as to the probability of this relationship being present (even though Dordi attributed the change to liver damage rather than to anoxemia). This work was carried out to determine whether any relationship existed between anoxemia *per se* and the guanidine content of the blood.

EXPERIMENTAL

Dogs were used as experimental animals. Only large, or moderately large, animals were selected to minimize as far as possible any error due to the slight degree of anemia resulting from the withdrawal of the relatively large blood samples (15 c.c.). The animals were fasted twenty-four hours before the experiment began and were not even allowed water during the course of the procedure.

Anoxemia was produced by the use of a tank in which the pressure and temperature could be held quite constant. The altitude in feet was computed by the barometric pressure inside the tank; likewise the partial pressure of oxygen. The period of anoxemia varied from thirty minutes to over seven and

TABLE I
BLOOD GUANIDINE IN ANOXEMIA

ANIMAL NO.	DATE	ALTITUDE IN FEET	PARTIAL PRESSURE OF O ₂ MM. HG	TIME OF ANOXEMIA	UREA N MG. %	SUGAR MG. %	GUANIDINE MG. %	CO ₂ CAPACITY VOL. %	HEMOGLOBIN %	CRIT. VOL. %
<i>A. Using Separate Dog as Control</i>										
1a	3/ 5/39	Control	150	None	12.9	154	0.68	39	15.8	
1b		18,000	80	45 min.	19.4	126	0.78	76	18.1	
2a	3/10/39	Control	150	None	12.6	156	0.31	37	11.9	
2b		18,000	80	30 min	8.1	136	0.34	39	13.0	
3a	3/14/39	Control	150	None	17.2	118	0.31	52	11.0	
3b		18,000	80	30 min	17.4	125	0.45	49	14.8	
4a	3/16/39	Control	150	None	16.4	122	0.44	34	16.4	
4b		24,000	63	30 min	21.6	136	0.63	42	18.7	
5a	3/18/39	Control	150	None	9.9	145	0.28	34	15.7	
5b		24,000	63	30 min	10.8	152	0.56	36	18.5	
6a	3/23/39	Control	150	None	15.7	152	0.61	44	16.5	
6b		24,000	63	30 min	13.3	143	0.36	38	14.7	
7a	4/ 8/39	Control	150	None	9.5	204	0.59	38	15.1	
7b		28,000	53	30 min	11.0	172	0.68	24	14.8	
8a	4/14/39	Control	150	None	15.0	105	0.56	33	16.4	
8b		28,000	53	30 min	14.0	159	0.57	44	16.3	
Average		Control anox-emic			13.5 16.2	151 144	0.47 0.55	39 44	14.8 16.1	
<i>B. Using Same Animal as Control</i>										
9	7/18/39	Control	150	None	13.3	74	0.70	36	12.5	36
		18,000	63	4 hr.	15.0	69	0.62	40	14.6	39
10	7/19/39	Control	150	None	9.8	105	0.67	40	14.5	39
		18,000	63	4 hr.	10.5	80	0.65	34	15.2	41
11	7/19/39	Control	150	None	23.1	74	0.59	41	17.1	45
		18,000	63	4 hr.	33.3	75	0.54	32	17.3	45
12	7/21/39	Control	150	None	9.8	67	0.74	29	14.9	40
		24,000	63	4 hr.	13.1	69	0.67	27	14.5	39
13	7/21/39	Control	150	None	11.0	77	0.68	42	15.8	41
		24,000	63	4 hr.	11.6	87	0.66	36	15.7	42
14	7/22/39	Control	150	None	13.3	74	0.70	36	12.8	36
		24,000	63	4½ hr.	21.0	81	0.68	28	14.7	38
15	7/24/39	Control	150	None	9.8	105	0.67	40	14.5	39
		24,000	63	7½ hr.	12.1	86	0.81	21	15.5	41
16	7/24/39	Control	150	None	23.1	74	0.59	41	17.1	45
		24,000	63	7½ hr.	18.4	83	0.64	35	17.5	46
Average (Nos. 9-16)		Control anox-emic			14.2 17.0	79 82	0.66 0.66	38 32	14.9 15.6	40 42
Average of all		Control anox-emic			13.3 16.0	113 111	0.57 0.60	39 38	14.9 15.9	41 42
		Change			+3.7	--	+0.03	--	1.0	42

one-half hours. The altitudes used varied from 18,000 to 24,000 feet (oxygen pressures of 80 to 53 mm. Hg, respectively). It is believed that this degree of anoxemia (especially in the seven-and-one-half-hour periods) is entirely ample to bring out any alterations in the blood chemistry that might be observed in life during anoxemia resulting from a spastic state.

In the first group of animals two dogs were used for each experiment: one as a control and one for the anoxemic experiment. Both dogs were anesthetized with sodium barbital and sacrificed after the experiment. The use of a separate control was decided upon to eliminate the possible error from blood loss due to the withdrawal of blood samples. The anesthetic served to obviate errors arising from muscular activity and dehydration during the period of anoxemia. In the second group of animals the same dog was used as a control, that is, samples were withdrawn before and after the period of anoxemia. No anesthetic was employed in this group and only relatively large animals were chosen.

Blood sugar, urea, and carbon dioxide capacity were determined by standard analytical procedures. Blood guanidine was estimated by the method of Andes and Myers.²⁴ Hemoglobin was measured by both photoelectric and colorimetric procedures. The determinations were all made immediately after the blood was withdrawn. The results are given in Table I. The table is divided into two parts in order to separate the two groups of animals.

DISCUSSION OF RESULTS

The findings are essentially negative in both groups of animals except for the blood urea, hemoglobin, and cell volume. The blood urea increase is significant although small. No explanation can be given for this change other than the effect of anoxemia on kidney function. The amount of hemoconcentration present, as denoted by the values for hemoglobin and cell volume, was too small to account for all this increase in the concentration of blood urea. The changes in the hemoglobin and cell volume were about what one would expect from the degree of anoxemia present. The values for guanidine and blood sugar were not significantly changed by the experiment. It will be noted that the glucose content of the blood is abnormally high in the group of animals in part A, Table I. This is presumably due to the fact that these animals received ether prior to the administration of barbital. The lack of further rise of blood sugar in the experimental animals may be due to the inhibiting effect of barbiturates on epinephrine release which would otherwise be produced by anoxia. The group of animals in part B, Table I received no anesthesia. Here again there is a lack of hyperglycemic response to anoxia. It must be pointed out, however, that the blood samples were drawn after four hours or more of anoxia.

The fall in the carbon dioxide capacity in the second set of animals is significant, but it is no more than one would expect from the amount of hyperpnea produced by the lack of oxygen and the additional hours of starvation. In the first group of animals the values for the carbon dioxide capacity are too erratic to be of any significance.

SUMMARY

1. Under the conditions described in this paper definite increases in the values of blood urea, hemoglobin, and volume of red blood cells were observed in anoxemic dogs. The guanidine content in the blood, however, was unchanged.

2. Some factor other than anoxemia must be sought as the cause of the hyperguanidinemia found in convulsive states.

REFERENCES

1. Andes, J. E., and Emerson, G. A.: Guanidine-Like Substances in Blood. VI. Blood Guanidine After Administering Convulsant Drugs, *Arch. internat. de pharmacodyn. et de therap.* 40: 30, 1938.
2. Andes, J. E., and Myers, V. C.: Guanidine-Like Substances in Blood. IV. Blood Guanidine in Patients With Parathyroid Deficiency and With Idiopathic Tetany, *J. LAB. & CLIN. MED.* 23: 123, 1937.
3. Berencsy, G., and Zinhobel, K.: Über einige gegen die Guanidintheorie sprechende Versuche, *Klin. Wchnschr.* 9: 1503, 1930.
4. Bryan, W. R., Minot, A. S., and Chantain, L. L.: Hyperguanidinemia Associated With Dehydration in Normal and Parathyroidectomized Dogs, *Am. J. Physiol.* 106: 735, 1933.
5. Major, R. H., Orr, T. G., and Weber, C. J.: Observations on the Blood Guanidine in Tetania Parathyreopriva, *Bull. Johns Hopkins Hosp.* 40: 287, 1927.
6. Saunders, J. A.: Guanidine and the Parathyroid Glands, *Biochem. J.* 29: 1597, 1935.
7. Swingle, W. W., and Nicholas, J. S.: The Effect of Chemical Compounds on the Production of the Tetany Syndrome, *Am. J. Physiol.* 69: 455, 1924.
8. von Beznak, A., and Szeöke, K.: Über den Zusammenhang von Guanidinstoffwechsel und Aktivität der Leberarginase bei parathyreopriv tetanischen Hunden, *Biochem. Ztschr.* 239: 159, 1931.
9. Laffont, A., Malmejac, R., and Sirjean: Guanidinemia and Gestation, *Ann. de méd. phys.* 6: 395, 1930.
10. Malmejac, J.: Guanidinémie et intoxication gravidique, *Rev. franç. de gynéc. et d'obst.* 25: 435, 1930.
11. Malmejac, R.: Les rapports de la guanidinémie avec la puerpéralité, *Gynéc. et obst.* 24: 689, 1931.
12. Minot, A. S., and Cutler, J. T.: Increase in Guanidine-Like Substance in Acute Liver Injury, *Proc. Soc. Exper. Biol. & Med.* 26: 607, 1929.
13. Andes, J. E., Andes, E. J., and Myers, V. C.: Guanidine-Like Substances in the Blood III. Blood Guanidine in Normal Pregnancy, Toxemias of Pregnancy, and Cirrhosis of the Liver, *J. LAB. & CLIN. MED.* 23: 9, 1937.
14. Traut, E. F., and McFate, R. P.: Hyperguanidinemia in Tetany, *J. A. M. A.* 96: 266, 1931.
15. de Wesselow, O. L. V. S., and Griffiths, W. J.: Blood Guanidine in Hypertension, *Brit J. Exper. Path.* 13: 428, 1932.
16. Stander, H. J.: Guanidine Base Concentration in Eclampsia, *Am. J. Obst. & Gynec.* 23: 373, 1932.
17. Krieger, V. I.: Observations on Guanidine, Glucose and Calcium Content of Blood in Eclampsia, *M. J. Australia* 2: 746, 1934.
18. Andes, J. E., Linegar, C. R., and Myers, V. C.: Guanidine-Like Substances in the Blood II. Blood Guanidine in Nitrogen Retention and Hypertension, *J. LAB. & CLIN. MED.* 22: 1209, 1937.
19. Ellis, M. M., Neal, M. P., and Frazier, T. R.: Guanidine Content of Blood From Epileptics, *Proc. Soc. Exper. Biol. & Med.* 28: 553, 1931.
20. Dodd, K., Minot, A. S., and Casparis, J.: Guanidine as a Factor in Alimentary Intoxication in Infants, *Am. J. Dis. Child.* 43: 1, 1932.
21. Dodd, K., and Minot, A. S.: Hyperguanidinemia and Tetany, *Am. J. Dis. Child.* 47: 958, 1934.
22. Schumacher, H. M.: Über die Beziehung des Guanidins zur Tetanie, *Ztschr. f. Kinderl.* 56: 307, 1934.
23. Dordi, A. M.: Guanidine in Blood in Diseases of the Respiratory Tract in Children, *Riv. di clin. pediat.* 37: 448, 1939.
24. Andes, J. E., and Myers, V. C.: Guanidine-Like Substances in Blood. I. Colorimetric Estimation and Normal Values, *J. LAB. & CLIN. MED.* 22: 1147, 1937.

A COMPARISON OF THE CHLORIDE CONCENTRATIONS IN WHOLE BLOOD AND SPINAL FLUID*

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IT IS generally believed that spinal fluid is formed from the fluids and solutes of the blood, and that the composition of the former medium reflects more or less closely that of the latter.¹ Comparative studies of these fluids have been extensively carried out in this country by Fremont-Smith and various co-workers.²⁻⁶ In part as a result of these and similar studies, the thesis has been developed that spinal fluid can be properly regarded as a modified dialysate of plasma. As this thesis has been adequately presented and discussed in various recent textbooks,⁷⁻⁹ a review of the history of the problem will not be given here.

The majority of the papers which have dealt with this subject have had for their purpose an examination of the evidence favoring or criticizing the thesis just stated. This has led previous investigators to carry out their determinations under certain specified conditions. Since the spinal fluid is apparently, at least in part, a dialysate of blood plasma,^{1, 10} precautions have been taken to secure specimens for analysis which shall resemble as closely as possible plasma as it exists in the body—the so-called “true plasma.” Blood has been collected without stasis under oil, and the cells removed from the specimen by centrifuging without contact with air. These precautions are taken to avoid changes in the carbon dioxide tension and the migration of chloride ions between the cells and plasma, that is, the so-called “chloride shift,” which results from changes in this tension.¹¹ Specimens of blood and fluid have regularly been drawn after a night's fast when the effects of the ingestion of food and water are at a minimum.

Since the determination of the chloride content of the spinal fluid has been found to be of value in the diagnosis of meningitis, particularly of tubercular meningitis,^{1, 14, 15} such analyses are frequently requested of clinical chemical laboratories. It seemed desirable to analyze blood and spinal fluid taken simultaneously in order to call the clinician's attention to the presence of a lowered chloride in the blood when such existed. This should decrease any tendency to attach too much specificity to the results of the spinal fluid analyses. It was hoped also that such a procedure would make it possible to detect the effect upon the spinal fluid chloride concentration of factors not associated with variations in the blood, such as changes in the protein concentration of the spinal fluid^{2, 4} and possibly of the replacement of chloride by lactic acid formed during the destruction of sugar by organisms and enzymes.²

Soon after this program was instituted it was found that these specimens of spinal fluid submitted for analysis frequently were taken from patients not in the postabsorptive state. It was also recognized that under the conditions existing in the clinic, the samples of blood obtained were as a rule not suitable

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for the preparation of "true plasma." The procedure was, therefore, definitely modified from that followed by Fremont-Smith and his co-workers. Specimens were drawn at any time during the day, and the whole blood was chosen as the material to be used for the control analyses. The method of Whitehorn¹⁸ was used for the determination of chlorides in both blood and fluid.

It was recognized that this procedure was not as well adapted to the study of the physiologic problem involved as was that employed by the investigator already cited. However, it was felt that one principal source of error, that due to variations in the concentration of red blood cells which are relatively low in spinal fluid chloride, could be controlled by routine blood counts. The other chief sources of error, variations in the chloride concentration in the red blood cells, and failure of the spinal fluid and blood to reach a condition of approximate equilibrium were necessarily ignored.

We were not able to find any data which might serve as a base line to determine the usual ratio between the whole blood and spinal fluid chloride concentrations. We have, therefore, undertaken to establish such a standard by a statistical analysis of our own material. Because we feel that other clinics are probably confronted by problems similar to our own, we are presenting the results for publication. In order to compare the relationship with that obtained under more nearly ideal conditions, we have analyzed the 331 determinations made upon true plasma and spinal fluid, obtained in the postabsorptive condition, published by Fremont-Smith and his co-workers.^{5,6} The character of the clinical material upon which these earlier studies were based seems to have been similar to that from the wards and out-patient department of the Buffalo General Hospital, but the number of normal cases was apparently somewhat greater in the series of the plasma determinations. To facilitate comparison of the two sets of data the results have been expressed according to standard statistical methods.

Table I shows the distribution of the separate values for whole blood and spinal fluid of the 225 analyses made at the Buffalo General Hospital. Inspection of the table shows that there was a marked correlation between the chloride content of the two fluids in the various specimens. The values of the ratio $\frac{\text{spinal fluid chloride}}{\text{whole blood chloride}}$ are given in the table. These were neither absolutely constant nor wholly independent of the blood and spinal fluid chlorides. They apparently tended to increase slightly as the concentration of chloride in the spinal fluid increased, and to decrease as the concentration in the blood increased. The data on the chloride content of the plasma and the spinal fluid presented in the papers of Fremont-Smith and his co-workers are given in Table IA. It is evident that the range of values is much less than that shown by our own results. The difference between the ranges of the whole blood and of the plasma figures is readily explained, for the composition of the plasma is much more nearly constant than is that of blood, as variations in the number of red blood cells, containing relatively small amounts of chloride, do not affect the latter figure. Two factors appear to have produced the difference between the distributions of the spinal fluid values in the two series. One of these probably was a difference in the method of selection of the cases studied; the other was

TABLE I

WHOLE BLOOD CHLORIDE AND SPINAL FLUID CHLORIDE VALUES—225 DETERMINATIONS
(Buffalo General Hospital)

The values of the ratio $\frac{\text{spinal fluid chloride}}{\text{whole blood chloride}}$ have been multiplied by 100 and expressed as per cent.

SPINAL FLUID MG. NaCl/100 C.C. RANGE	WHOLE BLOOD—MG. NaCl PER 100 C.C.—RANGE OF VALUES								RATIO PER CENT
	251-300	301-350	351-400	401-450	451-500	501-550	551-600	TOTAL	
401-450	0	2	0	0	0	0	0	2	142.5
451-500	2	1	1	0	0	0	0	4	157.5
501-550	0	4	3	1	0	0	0	8	156.3
551-600	0	2	14	9	0	0	0	25	151.2
601-650	1	0	10	23	3	1	0	38	149.1
651-700	0	0	2	36	36	2	0	76	152.0
701-750	0	0	1	19	36	5	0	61	155.7
751-800	0	0	0	5	4	0	1	10	163.5
801-850	0	0	0	0	0	0	0	0	--
851-900	0	0	0	0	0	0	0	0	--
901-950	0	0	0	0	0	0	1	1	156.0
Total	3	9	31	93	79	8	2	225	
Ratio per cent	190.0	157.0	152.7	156.3	148.9	134.3	143.5		153.6

TABLE IA

PLASMA CHLORIDE AND SPINAL FLUID CHLORIDE VALUES—331 DETERMINATIONS
(Fremont-Smith and Co-workers)

The values of the ratio $\frac{\text{spinal fluid chloride}}{\text{plasma chloride}}$ have been multiplied by 100 and expressed as per cent.

SPINAL FLUID MG. NaCl/100 C.C. RANGE	PLASMA—MG. NaCl PER 100 C.C.—RANGE OF VALUES								RATIO PER CENT
	310-304	365-413	414-462	463-511	512-560	561-609	610-658	TOTAL	
498-546	0	0	2	0	1	0	0	3	114.8
547-595	1	1	0	7	3	0	0	12	122.7
596-644	0	0	1	8	17	1	0	27	119.8
645-693	0	0	1	6	34	33	1	75	123.4
694-742	0	0	0	2	11	163	21	197	122.7
743-791	0	0	0	0	0	7	10	17	123.3
Total	1	1	4	23	66	204	32	331	
Ratio per cent	171.5	151.5	133.5	126.5	122.4	122.2	122.2		122.6

fact that Fremont-Smith's studies were made upon specimens obtained in the postabsorptive state when there is relatively little variation in the composition of all body fluids. In our own series the effect of the ingestion of water and of salt was unquestionably marked in some instances. Except for the difference in the "spread" of the figures, Table IA bears a close resemblance to Table I. The correlation between the plasma and spinal fluid chloride values is marked, and the values of the ratio $\frac{\text{spinal fluid chloride}}{\text{plasma chloride}}$ tend to vary directly as the spinal fluid values, and inversely as the plasma chloride concentrations.

The correlation coefficients based upon these analyses are given in Table II. In both series the correlation between the chloride of the spinal fluid and the chloride of the plasma and of the whole blood was positive and marked. The parallelism between the spinal fluid and plasma chlorides was closer than was

TABLE II
CORRELATION COEFFICIENTS

	BLOOD OR PLASMA NaCl AND SPINAL FLUID NaCl	BLOOD OR PLASMA NaCl AND RATIO	SPINAL FLUID NaCl AND RATIO
Fremont-Smith Plasma 331 determinations	$+0.811 \pm 0.013$	-0.467 ± 0.029	$+0.091 \pm 0.037$
Hubbard and Beck Whole blood 225 determinations	$+0.766 \pm 0.018$	-0.483 ± 0.051	$+0.118 \pm 0.044$

that between the spinal fluid and the whole blood chlorides, as would be expected from the probable mechanism involved for the spinal fluid is apparently formed directly from the plasma. The existence of a slight positive correlation between the values of the ratio $\frac{\text{spinal fluid chloride}}{\text{whole blood (or plasma) chloride}}$ and of spinal fluid chloride suggested in Tables I and IA is confirmed to some extent by the correlation coefficients. Analysis of both series of data gave slight positive coefficients, which were probably, but not certainly, significant. We believe that this parallelism results from the relationship between the amount of protein and of chloride in the spinal fluid discussed previously by Merritt and Fremont-Smith,⁹ and shown for our own data in Table III. If the protein concentration affects directly the spinal fluid chloride, and is itself independent of variations in the chloride of the blood and plasma, values of the ratio should parallel values of the spinal fluid chloride, i.e., variations in the spinal fluid chloride will to some extent, be independent of variations in the blood chloride concentration.

Table II also shows that a significant negative correlation existed between the values of the ratio and the chloride concentration of the whole blood or plasma. While a slight negative correlation might arise from the method by which the ratio is calculated (for increases in the denominator of a fraction must have some tendency to cause decreases of the value of the fraction), it seems to us that the correlation is too close to be explained wholly in this way. We believe that variations in the plasma and the whole blood chloride sometimes do not cause changes of the usual magnitude in the concentration of that substance in the spinal fluid. One factor, and obviously an important one, has been discussed by Fremont-Smith.⁵ He suggested that it takes a fairly long period of time to establish an equilibrium between the chlorides in the blood and the spinal fluid. This was in accord with previous work, for it had already been shown that an equilibrium between the glucose concentration in the two fluids is reached rather slowly.¹⁵ He pointed out that the most pronounced divergence in values of the ratio from those expected occurred in patients who were losing chloride and water by emesis or who were receiving either chloride or water therapy. In our own series the same type of patient showed similar unusual relationships. It was noted, too, that in many instances ratios which deviated markedly from the average of the series were obtained on individuals who were showing marked variations in the chloride content of the spinal fluid. Such an "abnormal" ratio was not infrequently preceded or followed by an entirely "normal" ratio within one or two days. This strongly suggests that some delay in attaining equilibrium

TABLE III

CORRELATION COEFFICIENTS

COMPARISON OF SPINAL FLUID PROTEIN WITH BLOOD AND SPINAL FLUID CHLORIDES
(Hubbard and Beck—58 Determinations)

BLOOD NaCl AND SPINAL FLUID NaCl	BLOOD NaCl AND RATIO	SPINAL FLUID NaCl AND RATIO	SPINAL FLUID PROTEIN AND BLOOD NaCl	SPINAL FLUID PROTEIN AND SPINAL FLUID NaCl	SPINAL FLUID PROTEIN AND RATIO
+0.812 ± 0.030	-0.431 ± 0.072	+0.187 ± 0.084	+0.040 ± 0.080	-0.309 ± 0.080	-0.416 ± 0.073

TABLE IV

STATISTICAL CONSTANTS

Results of analyses made upon whole blood, plasma, and spinal fluid are expressed as milligrams of sodium chloride per 100 c.c.

SOURCE OF DATA AND FACTORS	MEAN MG./100 C.C.	MEDIAN MG./100 C.C.	STANDARD DEVIATION MG./100 C.C.	COEFFICIENT OF VARIATION PER CENT
Remont-Smith—331 specimens				
Plasma NaCl	570.3 ± 1.5	578.6 ± 1.9	40.80 ± 1.07	7.15 ± 0.19
Spinal fluid NaCl	698.0 ± 1.6	713.6 ± 2.0	43.17 ± 1.13	6.18 ± 0.16
Ratio $\frac{\text{spinal fluid NaCl}}{\text{plasma NaCl}}$	1.226 ± 0.002	1.224 ± 0.003	0.0622 ± 0.0016	5.08 ± 0.13
Hubbard and Beck—225 specimens				
Whole blood NaCl	433.9 ± 2.2	442.1 ± 2.7	48.58 ± 1.54	11.19 ± 0.36
Spinal fluid NaCl	664.1 ± 3.0	682.5 ± 3.7	66.41 ± 2.11	10.00 ± 0.32
Ratio $\frac{\text{spinal fluid NaCl}}{\text{whole blood NaCl}}$	1.536 ± 0.005	1.525 ± 0.008	0.1187 ± 0.0034	7.73 ± 0.25
Spinal fluid protein—58 specimens				
Whole blood NaCl	423.6 ± 5.0	430.5 ± 6.25	56.46 ± 3.53	13.33 ± 0.84
Spinal fluid NaCl	642.7 ± 7.4	649.1 ± 9.3	76.41 ± 4.80	11.89 ± 0.74
Spinal fluid protein	0.224 ± 0.021	0.105 ± 0.026	0.237 ± 0.015	104.29 ± 6.53
Ratio $\frac{\text{spinal fluid NaCl}}{\text{blood NaCl}}$	1.522 ± 0.012	1.498 ± 0.015	0.1372 ± 0.0086	9.01 ± 0.58

had affected the results. It is, of course, not possible to decide whether the occurrence of an unusual relationship between the chlorides in blood and spinal fluid always result from such a simple cause as this, or whether more specific factors must sometimes be considered in explaining the lack of constancy of the ratio $\frac{\text{spinal fluid chloride}}{\text{blood (or plasma) chloride}}$.¹⁰

It has been stated that there is evidence that the protein content of the spinal fluid has a direct effect upon the concentration of the chloride. Protein determinations were made by the method of Hubbard and Garbutt¹⁸ upon 58 of the 225 specimens of spinal fluid upon which this study is based. A statistical analysis of the results is shown in Table III. The correlation coefficients of the small series do not differ greatly from those of the larger one of which it forms a part. There was no relationship between the protein in the spinal fluid and the concentration of chloride in the blood. There were negative correlations between the spinal fluid protein on the one hand and the spinal fluid chloride and the values of the ratio $\frac{\text{spinal fluid chloride}}{\text{whole blood chloride}}$

on the other hand. Such a relationship would be expected if the spinal fluid protein causes, or is fairly regularly accompanied by, decreases in the spinal fluid chloride. Such decreases may result from a diminution of the usual difference between the protein concentrations of plasma and spinal fluid.'

The usual statistical constants for the figures obtained in our own series, for those calculated from the data of Fremont-Smith, and for the 58 cases upon which protein analyses were available are given in Table IV. There is need of only brief comment upon this table. The blood and spinal fluid chloride values showed a high degree of variability and gave distribution curves which departed markedly from the symmetrical form, as shown by the large difference between the mean and median values. The ratios calculated from these observed data showed relatively little variability, and, while not perfectly symmetrical, were nearly so. As expected, the values of ratios calculated from the data of Fremont-Smith, based upon analyses of true plasma, appeared to be more nearly constant and to show more symmetrical distribution than did those of ours based upon

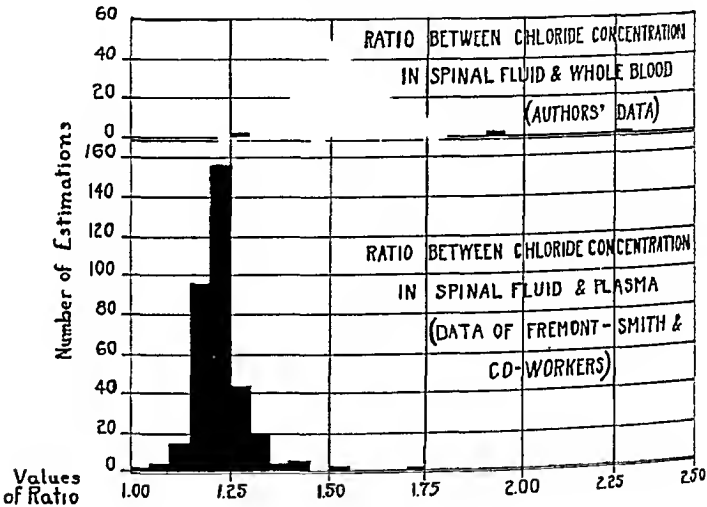


Chart 1.

determinations of the whole blood chloride. The difference between the two sets of values was not as great, however, as we had expected it would be.

Since it is hoped that the results of this study will be of value to other clinics in which spinal fluid analyses are carried out, Chart 1 has been prepared to show the distribution of the values of the ratios $\frac{\text{spinal fluid chloride}}{\text{whole blood chloride}}$ and $\frac{\text{spinal fluid chloride}}{\text{plasma chloride}}$. The chart shows graphically the distribution indicated in statistical terms in Table IV. These ratios show such constancy that either may be useful in checking the probable significance of spinal fluid analyses. The results based upon studies of true plasma were obviously somewhat more satisfactory than were those based upon analyses of whole blood.

CONCLUSIONS

Statistical analysis of a series of simultaneous determinations of whole blood chloride and spinal fluid chloride shows that a high degree of correlation exists

between the two sets of values. While the agreement of the spinal fluid chloride with the whole blood chloride was not as close as was the agreement of the former with plasma concentration, the agreement was nevertheless close enough to show that simultaneous determinations of the chloride content of whole blood can serve as a useful check upon the significance of spinal fluid analyses. Such controls should be especially valuable in clinics where the more complicated procedures cannot be readily carried out. It could be shown that three factors influence the value of the ratio between spinal fluid and whole blood chloride: (1) the protein content of the spinal fluid; (2) the concentration of cells in the blood; (3) the failure of the blood and spinal fluid to reach equilibrium when the fluid and salt content of the body were rapidly changing. Whether any other factor or factors influence the equilibrium, and so specifically affect the concentration of chloride in the spinal fluid, could not be determined.

REFERENCES

1. Mesetrat, W.: *Le liquide cephalo-rachidien*, Paris, 1912, A. Maloine et fils.
2. Fremont-Smith, F., and Dailey, M. E.: Cerebrospinal Fluid Chlorides, *Arch. Neurol. & Psych.* 14: 509, 1925.
3. Fremont-Smith, F., and Dailey, M. E.: The Effect of Protein Concentration Upon the Chloride Equilibrium Between Plasma and Cerebrospinal Fluid, *J. Pharmacol. & Exper. Therap.* 27: 255, 1926.
4. Fremont-Smith, F., and Dailey, M. E.: Studies in the Distribution of Chloride and Protein Between Plasma and Synovial Fluid, *J. Biol. Chem.* 70: 779, 1926.
5. Fremont-Smith, F., and Dailey, M. E., Merritt, H. H., Carroll, M. P., and Thomas, G. W.: The Equilibrium Between Blood and Spinal Fluid. I. The Composition of the Human Cerebrospinal Fluid and Plasma, *Arch. Neurol. & Psych.* 25: 1271, 1931.
6. Fremont-Smith, F., and Dailey, M. E., Merritt, H. H., and Carroll, M. P.: The Equilibrium Between Cerebrospinal Fluid and Blood Plasma. II. The Composition of the Human Cerebrospinal Fluid and Blood Plasma in Meningitis, *Arch. Neurol. & Psych.* 25: 1290, 1931.
7. Peters, J. P., and Van Slyke, D. D.: *Quantitative Clinical Chemistry*, Vol. I. Interpretations, Baltimore, 1931, p. 1075.
8. Katzenelenbogen, S.: *The Cerebrospinal Fluid and Its Relation to the Blood*, Baltimore, 1935, p. 101.
9. Merritt, H. H., and Fremont-Smith, F.: *The Cerebrospinal Fluid*, Philadelphia, 1937, p. 20.
10. Fremont-Smith, F.: The Nature of Cerebrospinal Fluid, *Arch. Neurol. & Psych.* 17: 317, 1927.
11. Best, C. H., and Taylor, M. B.: *The Physiological Basis of Medical Practice*, Baltimore, 1937, p. 168.
12. Fremont-Smith, F., and Dailey, M. E.: Cerebrospinal Fluid Sugar, *Arch. Neurol. & Psych.* 14: 390, 1925.
13. Hubbard, R. S., and Allison, C. B.: An Apparent Effect of Normal Variations in the Respiratory Rate Upon the Excretion of Chloride and Water, *J. Biol. Chem.* 89: 627, 1930.
14. Nobecourt, P., and Voisin, R.: Les chlorures de liquide cephalo-rachidien dans divers etats pathologiques et en particulier dans le bronchopneumonies infantile, *Arch. gén. de méd.* 2: 3018, 1903.
15. Neuda, P.: Die praktische und theoretische Bedeutung der Chlorbestimmung im liquor cerebrospinalis, *Wien. klin. Wchnschr.* 36: 447, 1923.
16. Whitehorn, J. C.: System of Blood Analysis, *J. Biol. Chem.* 45: 449, 1921.
17. Fremont-Smith, F., and Dailey, M. E.: Cerebrospinal Fluid Sugar, *Arch. Neurol. & Psych.* 14: 390, 1925.
18. Hubbard, R. S., and Garbutt, H. R.: The Determination of Protein in Cerebrospinal Fluid, *Am. J. Clin. Pathol.* 5: 433, 1935.
19. Best, C. H., and Taylor, N. B.: *The Physiological Basis of Medical Practice*, Baltimore, 1937, p. 1492.

LABORATORY METHODS

GENERAL

A STANDARDIZED TECHNIQUE FOR SEDIMENTATION RATE*

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LACK of a universally accepted technique for recording erythrocyte sedimentation rate has been a serious block to a more widespread adoption of this simple test in general practice. The great majority of techniques described have complicated the problem, making comparison of results by the various procedures difficult.

All techniques described were originally developed empirically. In each the unit of comparison (usually the total drop at the end of one hour expressed in millimeters), while it reflects the rapidity of sedimentation in a general way, fails to give an accurate idea of the rate of settling. Furthermore, an arbitrary determination at the end of a stated interval, such as one hour, also reflects anemia because of the packing of the cells in the bottom of the tube within the first hour in rapidly settling blood. Efforts to "correct" for anemia, as advocated by some investigators, have failed to yield consistent sedimentation findings.

The object of the present paper is to describe a technique that is based on principles applicable to all tubes. The method is simple, and requires no correction for anemia. In a way this technique is a modification of the previous Cutler method, but the approach and interpretation are totally different. To understand it, it is necessary to keep in mind the present concept of the factors believed responsible for the rapid settling of the erythrocytes.

It is generally accepted that rapid sedimentation is dependent upon the formation of large aggregates or rouleaux of red blood cells and that rouleaux once formed settle at a given rate of speed, more or less in accordance with Stokes' law of hydrodynamics—the larger the aggregate the more rapid the settling and vice versa (Figs. 1 and 2). It has been established that the arrangement of the red blood cells in rouleaux and the size of the rouleaux are effects brought about almost entirely by the plasma and are specific for that plasma, and that the size and number of cells in suspension, as in anemia, have little influence on this specificity of the plasma.¹ The exact nature of the changes in the plasma which bring about the formation of large rouleaux is not known, but it is generally believed that the hydrophilic substances present in the plasma, i.e., the proteins which vary in type and electrolyte combination, play

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a predominant role, probably through surface dehydration or change in the water balance on the surface of the red blood cells.

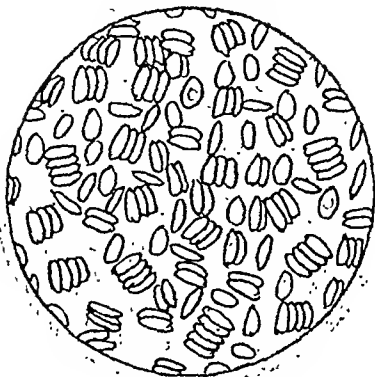


Fig. 1.—Rouleaux formation in slowly settling blood. The rouleaux are small and consist of relatively few cells.

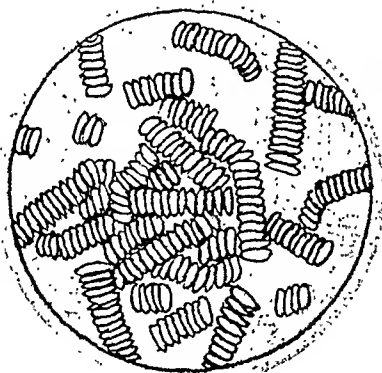


Fig. 2.—Rouleaux formation in rapidly settling blood. The rouleaux are large and consist of many cells. It is the increased mass of the large rouleaux that brings about rapid sedimentation. This fundamental fact underlies the technique for determining sedimentation rate described in the text.

THREE PHASES OF ERYTHROCYTE SEDIMENTATION

We are now in a position to study in detail the sedimentation phenomenon as recorded in the form of graphs. Four distinct types of graphs, a horizontal

line, a diagonal line, a diagonal curve, and a vertical curve (Fig. 3), have been recognized.² The horizontal line is normal and denotes slow sedimentation. The other graphs are abnormal findings and indicate various degrees of increased sedimentation. The vertical curve (Fig. 3D), which indicates the most rapid form of sedimentation, consists of three phases. The first is a slow phase, during which the cells are grouping themselves and forming aggregates. This aggregation period usually varies from five to fifteen minutes; occasionally it is as long as thirty minutes. Once formed, the aggregates fall uniformly, more or less in accordance with Stokes' law—the larger the aggregate the more rapid the fall. This is the second or settling phase and gives rise to the sedimentation phenomenon.

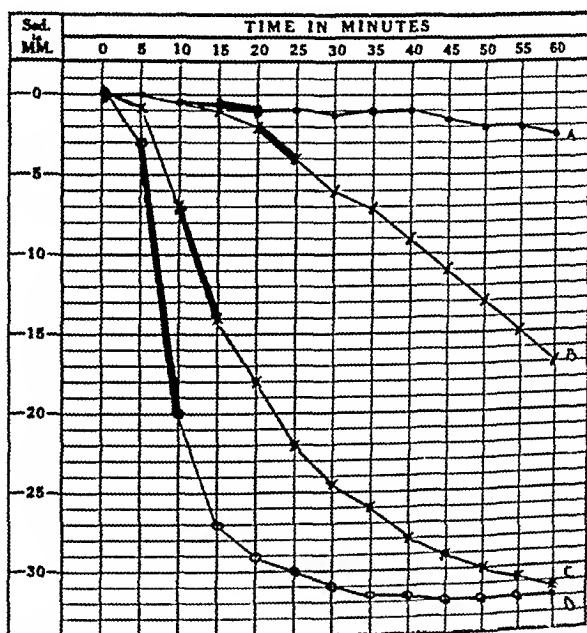


Fig. 3.—Typical graphs depicting erythrocyte sedimentation. A, horizontal line; B, diagonal line; C, diagonal curve; D, vertical curve. Only the horizontal line is normal. When the retarding influence of packing is absent, the rate of settling of the rouleaux once formed, is fairly uniform; this is best illustrated in graph B, where the rate is 2 mm. every five minutes.

The heavy part of each graph denotes the maximum settling in any five minutes during the hour and is a better guide to sedimentation rate than the sedimentation index (total drop at the end of one hour). Thus, graphs C and D have practically the same index (32 and 24 mm., respectively) but strikingly different maximum rates in five minutes (7 and 17 mm., respectively). Graph C is from a patient in her ninth month of pregnancy; graph D is from a patient with fatal carcinoma of the lung. Note that the maximum sedimentation rate in five minutes almost invariably occurs during the first thirty minutes and that no readings need be taken with the new technique beyond that time.

The aggregates settle at their respective speeds indefinitely, depending on the length of the tube, until they reach bottom. The aggregates that follow pack on top, and the packing stage, or third phase, of sedimentation sets in. Sedimentation during the packing stage is always slow. When all aggregates have settled tightly, sedimentation comes to a standstill.

It is apparent that by the time packing ensues, the sedimentation phenomenon has already taken place and that the packing of the cells represents only a rough hematocrit finding, recording the space in the tube occupied by the cells. The packing stage is the only phase of sedimentation that is ma-

terially influenced by the degree of anemia. The fewer the cells, the less volume they occupy.

The three phases, aggregation, sedimentation, and packing, are found in all graphs, in the horizontal normal as well as in the definitely abnormal vertical curve. The difference is in the time required to complete the second stage, or stage of sedimentation which depends upon the mass of the aggregates formed during the first or aggregation phase, which in turn is a function of the plasma. To study the sedimentation phenomenon, one should be concerned principally with the second phase, or phase of settling.

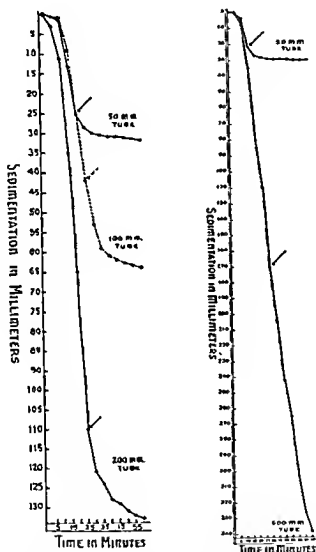


Fig. 4.—The influence of packing of the cells in the bottom of the tube (packing) on the rapidity of sedimentation in tubes 50, 100, 200, and 600 mm. long, respectively, and 5 mm. internal diameter. Readings every five minutes during the first hour (same sample of blood). In the first ten or fifteen minutes settling is slow in all tubes, for it is during this period that the red blood cells group themselves into rouleaux. Once rouleaux are formed, however, settling becomes rapid. Sedimentation reaches maximum 12 mm. in five minutes in the 50 mm. tube between fifteen and twenty minutes; 17 mm. in five minutes in the 100 mm. tube between twenty and twenty-five minutes; 45 mm. in five minutes between twenty and twenty-five minutes in the 200 mm. tube; and 50 mm. in five minutes between twenty-five and thirty minutes in the 600 mm. tube. From that point on (Indicated by arrows) sedimentation in five-minute periods becomes progressively slower in each tube because of the retarding influence of packing. Obviously the packing factor cannot be eliminated in tubes even 600 mm. long and no advantage whatever is gained in employing tubes longer than 50 mm. On the contrary, there are many disadvantages.

SEDIMENTATION RATE OF ROULEAUX

The object in the present technique was to determine the rate of sedimentation of the rouleaux during the phase of settling at a time when the rouleaux are formed and settling at a uniform rate of speed, uninfluenced by the period of aggregation of the red blood cells into rouleaux on the one hand, or their packing in the bottom of the tube on the other. Unfortunately, it is impossible

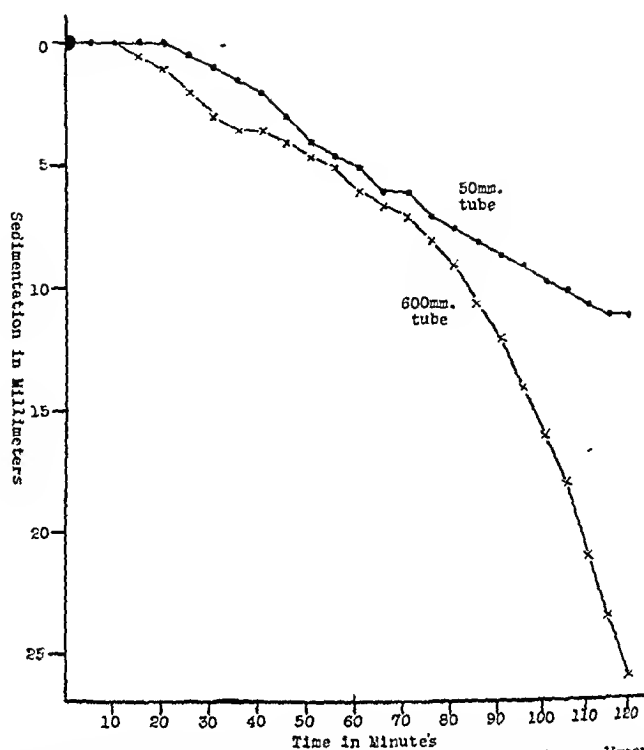


Fig. 5.—The length of tube is a decided factor in determining sedimentation rate, and this fact indicates the need for the adoption of a standard technique. The maximum drop in any five minutes in the 50 mm. tube is 1 mm.; in the 600 mm. tube, it is 2.5 mm. (same blood sample). The maximum rate in five minutes is reached much later in the 600 mm. tube than in the shorter 50 mm. tube.

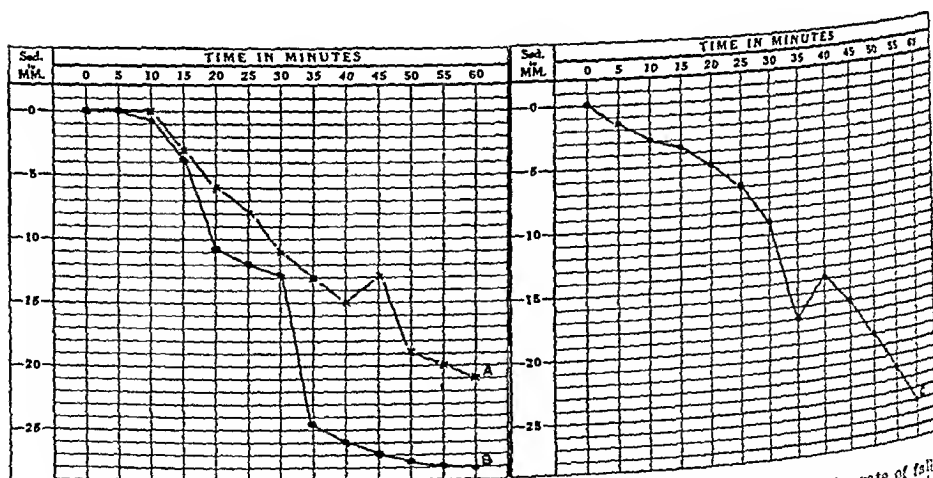


Fig. 6.—Bizarre sedimentation rate findings. Once rouleaux have formed, the rate of fall is fairly uniform. However, at times bizarre findings are observed, the explanation for which is not entirely clear, but they are in all probability mechanical in nature. It is worthy of note, however, that these bizarre records are self-correcting. Thus, if sedimentation is unduly slow in five minutes, the next reading will be unduly rapid (curve B), and vice versa (curve C). This again illustrates the unreliability of a single reading in determining sedimentation rate. The bizarre findings, while not common, were observed more frequently in tubes 100 and 200 mm. long than in 50 mm. tubes, and more commonly during the second half hour.

to eliminate the retarding influence of packing on sedimentation in rapidly settling blood in any tube of reasonable length, whether it be 50, 100, 200, or 600 mm. long (Fig. 4). In rapidly settling blood, the slowing effect of packing on sedimentation becomes evident well within the first hour, and the sedimentation rate is less in every one of the tubes mentioned than it would be normally in tubes of indefinite length. The only difference observed in tubes of 600 mm. or less in length was in the degree of slowing. It was also discovered that even in slowly settling blood, the rate of settling of the rouleaux, although slow in all tubes, is faster the longer the tube, and that the length of the tube is an important factor in determining sedimentation rate (Fig. 5). For practical reasons, therefore, it is necessary to determine the *maximum* sedimentation rate that the rouleaux can attain in a unit of time, e.g., five minutes, in a tube of fixed length. Such a unit would express the sedimentation velocity of the rouleaux at a time when it was *least* influenced by packing of the cells, and may be referred to as the maximum sedimentation rate or M.S.R., or simply sedimentation rate. Such a determination, for reasons mentioned above, would be independent of the anemia factor when present, and would be a true expression of sedimentation rate in that it denotes distance per unit of time and not distance at the end of a specified time as at present.

In the Cutler tube the maximum sedimentation rate in any five minutes can be determined during the first thirty minutes of settling, and no readings need be taken beyond that time, as the following study shows:

TABLE I

ANALYSIS OF 18 CASES (12.5% OUT OF A CONSECUTIVE SERIES OF 144) WITH SEDIMENTATION RATE FASTER IN A 5-MINUTE INTERVAL DURING THE SECOND 30 MINUTES OF SETTLING (CUTLER TUBE)

NO. OF CASES	M. S. R. IN ANY 5-MIN. PERIOD DURING FIRST 30 MIN. (MM.)	M. S. R. IN ANY 5-MIN. PERIOD DURING SECOND 30 MIN. (MM.)	DIFFERENCE (MM.)
3	0.5	1.0	0.5
2	1.0	1.5	0.5
1	2.0	2.5	0.5
1	0.5	1.5	1.0
3	1.0	2.0	1.0
2	2.0	3.0	1.0
1	3.5	4.5	1.0
1	2.0	3.5	1.5
1	5.0	7.0	2.0
1	2.0	4.5	2.5
1	4.0	7.0	3.0
1	2.5	6.0	3.5
18			

Only in the last three cases (2 per cent in this series) is the difference of some clinical significance.

Samples of blood from 144 consecutive patients were studied for their sedimentation rates by distributing parts of each sample into tubes 50, 100, and 200 mm. in length, respectively, and 5 mm. internal diameter. Readings were made every five minutes for one hour, and the results were recorded on sedimentation charts. (In an additional 93 cases simultaneous studies were made in 50 and 600 mm. tubes, respectively, but to simplify the presentation these are not discussed in detail.) The maximum settling in any five-minute period during the hour, after correcting for such bizarre findings as depicted in Fig. 6, was

determined for each sample of blood in each of the tubes studied. The results are recorded in Fig. 7. It will be noted that the M. S. R. made its appearance during the first thirty minutes of settling in the 50 mm. tube in 126 cases (87.5 per cent), in the 100 mm. tube in 126 cases (72.0 per cent), and in the 200 mm. tube in 81 cases (56.0 per cent). In the 600 mm. tube in many instances the M. S. R. did not make its appearance until the second hour of settling (Fig. 5).

Since a variation in the M. S. R. of 0.5 to 1 mm. is within normal limits,³ a critical analysis of the 18 cases (12.5 per cent) in the Cutler series (50 mm. tube) with a maximum sedimentation rate in five minutes greater during the second thirty minutes of settling (Table I), discloses that in only three cases, the last three in the table, was the difference of some clinical significance, but not sufficiently great to discredit the findings in the first thirty minutes. Thus there is in this series a potential error, of little importance clinically, of approximately 2 per cent (3 cases in a consecutive series of 144) by limiting all readings to the first half hour when determining the M. S. R. This is a much lower percentage of error than with tubes of greater length, and is a distinct advantage in using the Cutler tube.

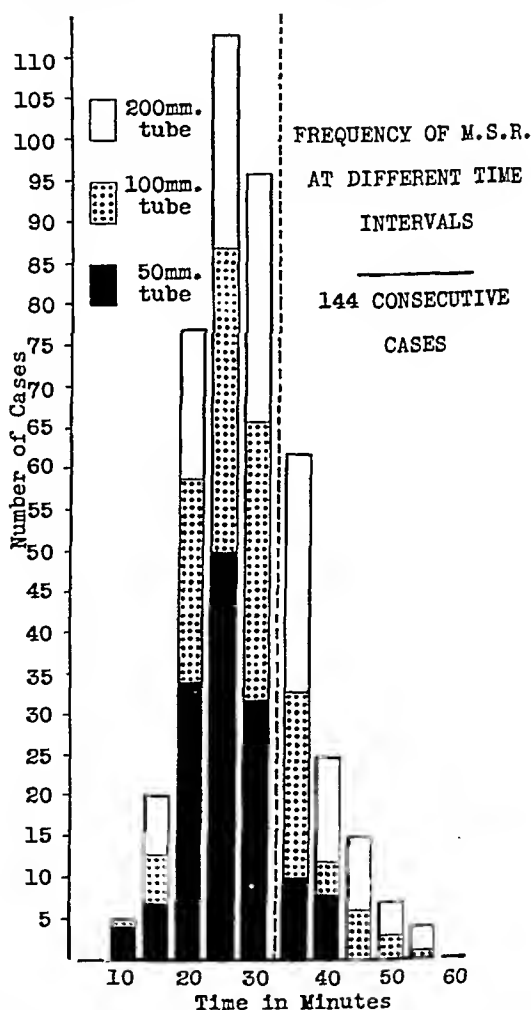


Fig. 7.—The maximum sedimentation rate (M.S.R.), expressed as the maximum settling in millimeters in any five minutes, was determined independently for each blood sample, in 50, 100, and 200 mm. tubes, respectively, and the frequency with which the M.S.R. occurred at different time intervals is recorded. In the 50 mm. tube the M.S.R. made its appearance in the first thirty minutes in 87.5 per cent of the blood samples studied (126 cases out of a consecutive 144). On the other hand, when the same bloods were studied in 100 mm. and 200 mm. tubes, the M.S.R. occurred in the first thirty minutes in only 72 and 56 per cent of cases, respectively. For details see text.

Other studies already completed³ are additional proof that the Cutler tube of 50 mm. length and 5 mm. internal diameter will give sedimentation rate information with less chance of error and with greater convenience and efficiency, because such technical factors as position of tube, have less effect than in longer tubes. It would seem justifiable, therefore, to agree on a tube of this length in an effort to standardize the technique.

DETAILS OF TECHNIQUE

The essential features of the new technique are as follows:

Cutler sedimentation tubes of 1 c.c. capacity are used, graduated into 50 mm. divisions, with 0 at the 1 c.c. level. One-tenth of 1 c.c. of 3.8 per cent sodium citrate solution (age does not matter) is aspirated into a 2 c.c. syringe and blood, obtained by puncture of a suitable vein, drawn to the 1 c.c. mark (for convenience, larger quantities of blood and citrate, but in the same proportion, may be used). After drawing back the barrel of the syringe about 1 cm., the blood and citrate are gently mixed by tilting the syringe backward and forward several times. The contents are then emptied into the sedimentation tube up to the zero mark, and the tube is placed in a special rack. The position of the sedimenting column of erythrocytes is determined every five minutes for one-half hour. The observations are recorded on charts that have been designed for the purpose on which the horizontal lines represent the divisions on the tube and the vertical lines the intervals of time. Graphs are then constructed, which not only show the position of the sedimenting column of erythrocytes at any period of time during the first half hour, but also portray changes in velocity that occur during the process of sedimentation. This leaves a permanent, visual record, easily comprehended and readily compared. The biggest drop in any five-minute period during the first half hour is the maximum sedimentation rate in five minutes (M.S.R.) that the rouleaux will attain in the Cutler tube, and becomes the unit of comparison.*

EVALUATION OF SEDIMENTATION RATE

The evaluation of sedimentation rate presented in this paper, expressed as the maximum settling of the rouleaux in five minutes during the first thirty minutes (Cutler tube), is based on an analysis of 1,068 consecutive cases. In each instance sedimentation readings were made every five minutes for one hour, and graphs were constructed. At the same time the maximum sedimentation rate in any five-minute period during the first thirty minutes of settling (M.S.R.) was determined for each graph. The graph and maximum sedimentation rate were then compared and a tabulation was made (Table II). Cases with a maximum sedimentation rate of 2 mm. or less were restudied as a separate group, and a detailed comparison was made between the maximum sedimentation rate and the sedimentation index (Table III).

It has been generally accepted that in the original Cutler technique a graph represented by a horizontal line is a normal sedimentation finding and that all

*In large institutions where it is desirable to include the sedimentation rate in the routine blood determinations and heavy laboratory schedules make immediate examination impractical, reliable and accurate results will be obtained by distributing tubes containing 0.2 c.c. of 3.8 per cent sodium citrate solution and having 2 c.c. of blood syringed into this container at the time blood is drawn for other determinations. This amount suffices for use in the 1 c.c. Cutler tube (50 mm. by 5 mm. column). The test may be performed at any time within four hours of citration. The time interval clock for announcing five-minute intervals will be found time-saving and practical.

other graphs are pathologic findings of increasing clinical significance. Thus a diagonal line with a sedimentation index of 15 mm. or less, although pathologic, is usually associated with disease of slight intensity, as a rule without clinical manifestations of toxemia; a diagonal line with an index of 16 mm. or more with disease of slight to moderate intensity, but accompanied as a rule by constitutional symptoms of slight degree; a diagonal curve, with disease of moderate intensity accompanied usually by toxemia of moderate degree; and a vertical curve, with disease of marked intensity, associated almost invariably with marked constitutional symptoms. In the same technique the sedimentation index represents the total sedimentation at the end of one hour expressed in millimeters. A sedimentation index of 8 mm. or less is considered normal; an index of 9 mm. or more, pathologic.

With these explanatory remarks the following interpretation may be placed upon the maximum sedimentation rate in five minutes (M.S.R.).

TABLE II

EVALUATION OF MAXIMUM SEDIMENTATION RATE IN TERMS OF SEDIMENTATION GRAPH
(BASED ON 1,068 CONSECUTIVE CASES)

There is a distinct correlation between maximum sedimentation in five minutes and sedimentation graph. H.L., horizontal line; D.L.—15, diagonal line, sedimentation index 15 mm. or less; D.L.—16, diagonal line, sedimentation index 16 mm. or more; D.C., diagonal curve; V.C., vertical curve; M. S. R. is expressed in millimeters.

SEDIMENTATION GRAPH

	NO. CASES	H.L.	D.L.—15	D.L.—16	D.C.	V.C.
		NO. CASES	NO. CASES	NO. CASES	NO. CASES	NO. CASES
	0.5	158	156	2		
	1.0 ^{1,2}	164	143	21		
	1.03-5	89	13	76		
	1.5	74	3	67	4	
	2.0	125		79	46	
	2.5	44		6	38	
	3.0	103		2	95	6
	3.5	33			16	17
	4.0	81			39	42
	5.0	55				55
	6.0	44				44
	7.0	31				31
	8.0	22				22
	9.0	15				15
	10.0	12				10
	11.0	8				1
	12.0	5				7
	13.0	1				5
	14.0	1				1
	15.0	1				1
	16.0					1
	17.0	1				
	18.0					
	19.0					
	20.0	1				1

Normal Sedimentation Rate.—In Table III are analyzed 610 cases in this study with a maximum sedimentation rate of 2 mm. or less. These are subdivided into five groups: (1) those with an M.S.R. of 0.5 mm. or less; (2) an M.S.R. of 1 mm., but with the 1 mm. rate not repeated more than twice during the first thirty minutes of settling, expressed in the tables as M.S.R.¹⁻²; (3) an M.S.R. of 1 mm., but with the 1 mm. rate repeated three or more times during the first

thirty minutes of settling, expressed in the tables as M.S.R.³⁻⁵; (4) an M.S.R. of 1.5 mm.; and (5) an M.S.R. of 2 mm. Each maximum sedimentation rate is compared with the corresponding sedimentation index as defined above, and a table of parallel values is constructed.

It is apparent from a study of Table III that only a maximum settling in five minutes of 1 mm. or less, with the maximum rate of 1 mm. in five minutes not repeated more than twice during the first thirty minutes of settling, is normal, and that only such values correspond to a sedimentation index of 8 mm. or less in the original Cutler technique. When the maximum sedimentation rate in five minutes is 1 mm., but the rate is repeated three or more times during the first thirty minutes, the rate, as judged by the sedimentation index, is increased and therefore pathologic.*

TABLE III

EVALUATION OF MAXIMUM SEDIMENTATION RATE IN TERMS OF SEDIMENTATION INDEX

Only a maximum sedimentation rate in five minutes (M. S. R.) of 1 mm. or less with the 1 mm. rate not repeated more than twice during the half hour is normal. It would not be grossly incorrect, however, in order to simplify matters, to speak of a maximum settling in five minutes of 1 mm. or less as normal, and anything more as abnormal.

MAXIMUM SEDIMENTATION RATE

Sedimentation Index (Cutler)	Normal	0.5	12	13-5	1.5	2
		NO. CASES	NO. CASES	NO. CASES	NO. CASES	NO. CASES
1		16	2	86%— index of 9 or more	95% have index of 9 or more	All have index of 9 or more
2		41	10			
3		34	17			
4		16	19			
5		16	16	1	1	
6		21	26	2		
7		7	30	1		
8		5	23	9		
9		2	14	10	5	3
10		99% have index of 8 or less	6	25	11	2
11			87% have index of 8 or less	20	8	6
12				13	18	8
13				5	12	16
14				1	5	22
15				2	8	23
16				14% have index of 8 or less	1	15
17						8
18					1	10
19					1	8
20					1	5

Increased Sedimentation Rate.—Table II compares the sedimentation graph with the corresponding maximum sedimentation rate, and permits the following evaluation of an increased maximum sedimentation rate:

(1) A maximum sedimentation rate of 1 mm. (repeated three or more times), but not greater than 2 mm., corresponds to a diagonal line with a sedimentation index of 15 mm. or less, and represents disease of slight intensity, usually without constitutional symptoms. (2) M.S.R. of 2.5 to 4 mm. corresponds to a diagonal line with an index of 16 or more and represents disease of slight to moderate intensity, usually associated with some clinical manifestations of toxemia. (3) A M.S.R. of 5 to 9 mm. corresponds to a diagonal curve

*The number of times the maximum rate in five minutes repeats during the first thirty minutes of settling gives additional and detailed information on the rapidity of sedimentation—the more times it repeats the more rapid the settling. To simplify matters, although not strictly correct, one can consider a maximum settling in five minutes of 1 mm. or less as normal, and anything more as abnormal.

and represents disease of moderate intensity, usually associated with moderate constitutional symptoms. (4) A M.S.R. of 10 mm. or more corresponds to a vertical curve and represents disease of marked intensity characterized, as a rule, by marked constitutional symptoms.

This indicates the requirements for precise determination and interpretation of the sedimentation rate. The unit maximum sedimentation rate in five minutes (M.S.R.) needs no correction for anemia; it cannot be misunderstood for it is determined objectively. It is important to remember that the sedimentation rate of the rouleaux is different in tubes of different length. Should one desire to use a tube longer than 50 mm. the same principles hold true, but a different scale must be set up. The different scales, however, have a definite relationship to each other and can be readily compared. Thus, compared with the 50 mm. tube, the rate is one-third faster in the 100 mm. tube, twice as fast in the 200 mm. tube, and three to four times faster in the 600 mm. tube, a ratio of approximately one-third increase in sedimentation for every 50 mm. increase in the length of the tube.

SUMMARY

A new technique, a modification of the Cutler, is presented for determining erythrocyte sedimentation rate; it is based on the fundamental fact that it is the size of the rouleaux that determines the rate of settling. This technique is precise, simple, and time-saving. The results are easy to interpret and need no correction for anemia. The method embodies principles that are applicable to all tubes. The essential features are as follows:

Cutler sedimentation tubes of 1 c.c. capacity are used, graduated into 1 mm. divisions, with 0 at the 1 c.c. level. One-tenth cubic centimeter of 3.8 per cent sodium citrate solution and 0.9 c.c. of blood obtained by puncture of a suitable vein are gently mixed in a 2 c.c. syringe and poured into the sedimentation tube, and the tube is placed in a special rack. The position of the sedimenting column of erythrocytes is determined every five minutes for one-half hour and is recorded on special charts. The maximum settling in any five minutes during the half hour is the sedimentation rate and becomes the unit of comparison.

For all practical purposes a maximum settling in five minutes of 1 mm. or less is normal. Everything else is abnormal and therefore pathologic. Generally speaking, maximum rates in five minutes between 1.5 and 4 mm. indicate disease of slight intensity; rates of 5 to 9 mm. indicate moderate intensity, and rates of 10 mm. or more, marked intensity.

Evidence is presented that the 1 c.c. Cutler tube is a practical tube for sedimentation rate determination, giving reliable and accurate results under average conditions. This tube appears suitable for standardization of technique.

The sedimentation charts may be obtained from Hartenstine Printing House, 206 DeKalb Street, Norristown, Pa.; the tubes, from Arthur H. Thomas Co., 230 S. 7th Street, Philadelphia, Pa.

REFERENCES

1. Cutler, J. W., Park, F. R., and Herr, B. S.: Influence of Anemia on Blood Sedimentation. *Am. J. M. Sc.* 195: 734, 1938.
2. Cutler, J. W.: Graphic Presentation of the Blood Sedimentation Test, *Am. J. M. Sc.* 171: 882, 1926.
3. Park, F. R., and Cutler, J. W.: Technical Factors Influencing Sedimentation Rate. In preparation.

RAPID DIAGNOSTIC METHOD FOR TESTING THE VIRULENCE OF CORYNEBACTERIA*

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VIRULENCE tests for the bacilli of the *Corynebacterium* group, carried out with pure cultures, require at least five to six days. While working with cultures of the *Corynebacteria* free from contaminating flora at the Laboratories of the Willard Parker Hospital, we succeeded in reducing to four days the time required for a report on the presence of toxin-producing diphtheria bacilli in swabs from the nose and throat. This was accomplished by culturing the material submitted for examination on a tellurite blood agar medium, and by using suspensions from pooled pickings of the *Corynebacteria* for virulence tests without subculturing them on other media.

Potassium tellurite, when incorporated in culture media in appropriate amounts, inhibits the growth of most pyogenic bacteria, without interfering with the growth of the bacilli of the *Corynebacterium* group (potassium tellurite media of Clauberg,¹ Pergola,² Anderson and co-workers,³ and Horgan and Marshall⁴). The colonies of the *Corynebacteria*, grown on these media from a material rich in pyogenic flora, reach in eighteen hours a greater size than on other media where they grow among numerous colonies of contaminants. Suspensions made from several colonies picked from tellurite media are heavy enough to be used for virulence tests. The few contaminants that have overcome the inhibitory action of the potassium tellurite, usually the staphylococci, are readily identified by the appearance of their colonies and, in doubtful cases, by means of smears.

METHOD

Culture Medium.—The medium used was a slightly modified one of Horgan and Marshall. The tellurite solution was prepared by autoclaving 1 Gm. of potassium tellurite in 100 c.c. of distilled water. Eight cubic centimeters of this solution mixed with 5 c.c. of defibrinated horse blood was kept in the icebox for from one to three days and then added to 100 c.c. of melted 2 per cent agar beef heart medium pH 7.4 to 7.6, at 50° to 60° C. The medium was distributed in Petri dishes. The plates remain good for about three weeks if kept in the icebox.

As the solubility of the potassium tellurite varies from one batch to another, and some of it may remain undissolved after autoclaving, the presumably 1 per cent solution from a new supply of the chemical was tested with a known culture of the diphtheria bacillus. If, after the use of 8 c.c. of the solution in

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the medium, the colonies of the diphtheria bacillus were small in eighteen hours, 7.5 c.c. was used instead, and the growth then was found to be satisfactory.

Preparation of the Suspension.—Swabs from the nose and throat were streaked on plates of the tellurite medium. The next morning the cultures were examined with a hand lens. Smears were made from portions of several colonies of various morphology, and stained with the Ponder stain in order to avoid inclusion of the staphylococci in the suspension. No attempt was made to identify the three types of the diphtheria bacillus or to exclude the diphtheroids. However, colonies of various morphology were included in the suspension. Five or more colonies of the *Corynebacteria*, picked off the plate, were suspended in 2 or 3 c.c. of broth which assumed a light gray color. When tested in Hopkins' tubes, the suspensions proved to contain 0.15 per cent of bacillary bodies.

Virulence Test.—Two-tenths cubic centimeter of the suspension was inoculated intracutaneously in the shaved abdomen of a guinea pig; three to four tests were made on one animal. A similar amount was injected intraperitoneally into another guinea pig which was used for the same number of control tests, and 500 units of diphtheria antitoxin was given subcutaneously. The guinea pigs were examined in 24, 48, and 72 hours. A black or purple area on the site of inoculation in the unprotected animal indicated the presence of a toxin-producing bacillus in the suspension. No discoloration and no inflammation of the skin was ever observed in the control animals.

In order to establish whether the results of the rapid virulence test were comparable with those obtained by the usual methods, 55 duplicate tests were made with suspensions of the *Corynebacterium* colonies grown on tellurite medium and with cultures of those bacteria isolated from ordinary blood agar plates.

Duplicate swabs were obtained from the material to be examined. One swab was streaked on the tellurite medium, and the suspension from the colonies of the *Corynebacteria* for the virulence test was made the next day, as described in the method; the final report on the virulence was ready on the fourth day. A duplicate swab was streaked on a plate of ordinary 5 per cent blood agar. In eighteen hours the colonies of the *Corynebacteria*, growing among the contaminants, were small, and those not contiguous with the colonies of the contaminants could not supply enough material for a bacterial suspension sufficiently heavy for the virulence test. Subcultures on Loeffler slants had to be made from these colonies, and from twenty-four to forty-eight hours more elapsed before a sufficient growth was secured for the bacterial suspension. The guinea pig was thus inoculated two or three days after the material had been received for the examination, and the virulence test was completed on the fifth or sixth day, or one or two days later than from tellurite plates.

In 54 cases the results of the virulence tests with the two bacterial suspensions were identical (28 cultures of toxic diphtheria bacilli, 26 cultures of non-toxic bacilli). In one case in which only 3 colonies of the *Corynebacteria* were available after eighteen hours' incubation on the tellurite medium, the suspension showed no toxin production, while the subculture from the blood plate revealed

the presence of toxin-producing bacilli. No discrepancy was observed when five or more colonies were used for the preparation of the suspension. Apparently, if the diphtheria bacilli are at all present in the material inoculated, at least one colony of toxin-producing bacilli is likely to be included in a suspension from five colonies.

The importance of pooling picked colonies for a single virulence test in order to avoid false negative results is mentioned by Fraser and MacNab.⁵

The incorporation of the potassium tellurite in the culture media does not affect the production of the toxin by the diphtheria bacilli, according to Clauser,⁶ Zopoth,⁷ and others.

No black discoloration of the guinea pig skin by the gray suspension of the tellurite-grown bacteria was ever observed in the antitoxin-protected animals. No discoloration other than toxic could, therefore, be expected in the nonprotected animals, and no false positive reactions were observed. However, we found it desirable to establish what amounts of the potassium tellurite might cause inflammation, necrosis, or discoloration of the skin. The use of 0.05 and 0.025 c.c. of a 1 per cent solution of potassium tellurite produced blackening of the site of intracutaneous injection; 0.015 c.c. produced a minute black dot, but smaller amounts left the skin unchanged. The amount of potassium tellurite in 0.2 c.c. of the bacterial suspensions used in the tests would be infinitely smaller than in 0.015 c.c. of a 1 per cent solution, so that no confusing discoloration of the guinea pig skin could be expected.

Suspensions of *Corynebacteria* grown on tellurite media were used occasionally for diagnostic virulence tests by other workers. Fraser and MacNab, in a review of the subject of virulence tests, mention in a brief paragraph the possibility of using these suspensions and indicate the need for pooling the suspensions from several colonies. Pavan,⁸ in a clinical article on the importance of virulence tests in patients and carriers of "modified diphtheria bacilli" indicates that crude (i.e., whole) cultures from slants of Pergola's medium have been used for bacterial suspensions.

While the use of *Corynebacteria* grown on tellurite media for the virulence tests is not new, its application to a four-day diagnostic test seems to be worth mentioning.

Four-day diagnostic virulence tests have heretofore been performed with suspensions from the original Loeffler slant growths ("field cultures"), as recommended by Havens and Powell.⁹ However, the presence of pyogenic contaminants in the washings from the crude cultures often produces a marked inflammation and suppuration at the site of intracutaneous inoculation, making the reading difficult.

Modified field cultures on Loeffler slants, with a diminished number of pyogenic contaminants, may be obtained by using subcultures from the rapid culture swabs of Brahdry, Lenarsky, Smith, and Gaffney.¹⁰ Their use also permits a four-day report on the virulence. However, this method requires subculturing after four hours. While very useful in an individual case, it is inconvenient if cultures continue to arrive at the laboratory at various hours of the day. Besides, some pyogenic bacteria usually get into the subculture.

The method of using picked colonies of the *Corynebacteria* from tellurite blood plates for the preparation of the bacterial suspensions, as recommended here, has the advantage of rapidity attained by the use of field cultures, combined with freedom from pyogenic microorganisms. In addition, there is the saving of labor and media by abolishing the need for subculture.

SUMMARY

A practical four-day diagnostic test for the virulence of bacilli of the *Corynebacterium* group is suggested.

The bacterial suspensions for guinea pig inoculation are prepared from colonies of the *Corynebacteria* cultured from the nose and throat swabs on blood agar potassium tellurite plates.

No false positive reactions are observed.

False negative results are avoided by the use of five or more colonies for the preparation of the bacterial suspensions.

We wish to thank Dr. Ralph S. Muckenfuss, Director of the Bureau of Laboratories of the New York City Health Department, for his advice in the preparation of this report.

REFERENCES

1. Clauberg, K. W.: Ueber einen Elektivnährboden für Diphtheriebazillen, der eine makroskopische Plattendiagnostik ermöglicht, *Zentralbl. f. Bakt., Abt. 1 Orig.* 114: 539, 1929.
2. Clauberg, K. W.: Weitere Mitteilung zur makroskopischen Diphtheriebazillendiagnostik, *Ibid.* 120: 324, 1931.
3. Pergola, M.: La differite, in *trattato italiano di igiene*, Torino, 1930, Unione tip. edit. Torinese, Vol. 6, Fasc. 4, p. 42.
4. Anderson, J. S., Happold, F. C., McLeod, J. W., and Thomson, J. G.: On the Existence of Two Forms of Diphtheria Bacillus—*B. diphtheriae* gravis and *B. diphtheriae* mitis and a New Medium for Their Differentiation and for the Bacteriological Diagnosis of Diphtheria, *J. Path. & Bact.* 34: 667, 1931.
5. Horgan, E. S., and Marshall, A.: A Simple Tellurite Medium for the Isolation of *C. diphtheriae*, *J. Hyg.* 32: 544, 1932.
6. Fraser, D. T., and MacNab, A. L. (Committee): Intracutaneous Virulence Test for *Corynebacterium diphtheriae*, *Am. J. Pub. Health* 27: 121 (Suppl., March), 1937.
7. Clauberg, K. W.: Ergänzennde Ausführungen über Brauchbarkeit, Herstellung und Verwendung der neuen Blutwasser-Glyzerolat-Tellur-Nährplatte zur bakteriologischen Diphtheriediagnostik, *Zentralbl. f. Bakt., Abt. 1 Orig.* 128: 153, 1933.
8. Zopoth, J.: La culture des bacilles diphthériques sur les milieux telluriques, *Compt. rend. Soc. de biol.* 121: 785, 1936.
9. Pavan, G.: Bacilli differici modificati e reazione intracutanea in cavia, *Gior. di batteriol. e immunol.* 20: 1148, 1938.
10. Havens, L. C., and Powell, H. M.: The Use of the Original Diagnostic Culture for the Determination of the Virulence of Diphtheria Bacilli, *Am. J. Hyg.* 2: 234, 1922.
11. Brahdry, M. B., Lenarsky, M., Smith, L. W., and Gaffney, C. A.: A Rapid Method for the Identification of Diphtheria Bacilli, *J. A. M. A.* 104: 1881, 1935.

TECHNIQUE AND COMPARATIVE VALUES OF CROSS AGGLUTINATION METHODS*

A STUDY OF CENTRIFUGE, SLIDE, AND HANGING-DROP TECHNIQUES

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THE cross-matching of recipient and donor bloods necessitates the use of some procedure which allows the mixing of cells and plasma (or serum) in such a way as to be read by the naked eye or microscope. For a long time the procedure in this hospital and in many others, the hanging-drop suspension was the method of choice. Of late we have used the thirty-minute open slide procedure with entirely satisfactory results.

In their article on "Intra-group Agglutinins in Blood Transfusions," Culbertson and Rateliff compared the hanging-drop method with the rapid centrifuge method of Landsteiner and found the latter to be distinctly more sensitive. On communication with Rateliff, we have learned that in using the hanging-drop method no specific effort was made to agitate the serum-cell mixture because this was technically impractical. We suspected from preliminary observations that the hanging-drop procedure was less sensitive because of the omission from the technique of some form of agitation. Shaking or some form of agitation is an essential part of any agglutination technique, the effect being apparently to throw particles or cells together mechanically, thus facilitating agglutination.

In order to prove this suspicion the following comparative studies on the centrifuge, hanging-drop, and open slide methods were carried out. Erythrocytes (II-A) from freshly drawn or bank blood, containing approximately 0.4 per cent sodium citrate, were washed three times in normal physiologic saline and a 5 per cent saline suspension was prepared. The titer of IV-0 plasma was then determined by each of the three methods.

The open slide method consists of thoroughly mixing a drop of cell suspension with several drops of plasma (or serum) on a ringed glass slide and allowing this to stand for thirty minutes under a Petri dish. A piece of moist filter paper is placed under the dish to prevent evaporation. The cells are thoroughly agitated by twirling and tilting the slide and the preparation is observed under the microscope.

The centrifuge method consists of shaking a few drops of plasma with one drop of washed cells for a minute and then incubating at 37° C. for five minutes. This is followed by centrifuging for three minutes and reading macroscopically. The hanging-drop method is similar to the open slide method,

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except that a hollow ground chamber and cover slip are used and the readings are made at the end of twenty minutes *without shaking*.

The sensitivity of the centrifuge method and open slide method was observed in the following procedure: Progressive geometric dilutions of plasma with saline were prepared (up to a dilution of 1:256) in 10 mm. test tubes. An equal size drop of the washed cell suspension was placed into all the tubes. Drops from each of these dilutions were placed on open glass slides, and the technique as described above followed. The remainder of the mixture in the test tubes was treated according to the centrifuge technique. This procedure enabled us to have the identical preparation in both set-ups. The results in Table I show that both methods were equally sensitive. Readings are microscopic.

TABLE I

PLASMA DILUTION	EXPERIMENTS 1		2		3		4		5	
	C.M.	SLIDE	C.M.	SLIDE	C.M.	SLIDE	C.M.	SLIDE	C.M.	SLIDE
0	4	4	4	4	4	4	4	4	4	4
2	4	4	4	4	4	4	4	4	4	4
4	4	4	4	4	4	4	3	4	4	4
8	3	3	3	4	2	2	3	3	4	4
16	2	2	2	2	1	1	3	2	4	3
32	1	2	2	2	1	1	0	1	3	2
64	1	1	1	1	0	1	0	1	3	1
128	0	1	1	1	1	0	0	0	1	0
256	0	0	0	0	0	0	0	0	0	0

The next procedure undertaken was to determine the comparative values with reference to the hanging-drop method. In all methods the cell plasma mixtures were identical for each dilution. Results are shown in Table II. Readings are microscopic.

TABLE II

PLASMA DILUTIONS	EXPERIMENT 1			EXPERIMENT 2		
	C.M.	SLIDE	H.D.	C.M.	SLIDE	H.D.
0	4	4	3	4	4	3
2	3	4	3	4	4	3
4	3	3	2	4	3	3
8	2	3	1	4	2	2
16	1	2	0	2	1	0
32	0	1	0	1	0	0
64	0	1	0	0	0	0
128	0	0	0	0	0	0
256	0	0	0	0	0	0

In comparing all three methods, one can readily see the close relationship of the centrifuge and the open slide methods, with some inferiority of the hanging-drop procedure. In preparing the cell-plasma mixture, the centrifuge method and the slide method permit shaking or agitation, a necessary requisite for any antigen antibody reaction. Both centrifuge and slide methods permit the application of this principle. With the hanging-drop preparation agitation is technically impractical, as it causes the suspension to flow to the line of glass contact, where observation is difficult. In several instances, agglutination was observed on the open slide method before and after agitating, and the titer was seen to rise two or three dilutions as a result of agitation.

We conclude, therefore, from our observations, that the centrifuge method and the open slide method have approximately the same degree of sensitivity, and that both are superior to the hanging-drop method. The centrifuge method has the advantage of requiring less time (ten minutes, as compared to the open slide method, forty minutes). The open slide procedure, however, facilitates the study of cold agglutination and pseudoagglutination.

REFERENCE

Culbertson, C. G., and Ratchiff, A. W.: Reaction Following Intra-Group Blood Transfusion, *Am. J. M. Sc.* 192: 471, 1936.

AN INEXPENSIVE CONSTANT TEMPERATURE PARAFFIN OVEN*

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DUE to the cost of constant temperature paraffin ovens some difficulty is experienced in obtaining a sufficient number for satisfactory operation of a technical laboratory. This difficulty has been met in our school by the construction of an inexpensive but efficient oven.

This oven consists of a wooden box, 28 inches long, 17 inches deep, and 7 inches high, which is fitted with a permanent pyramidal top tapering gradually to a height of 14 inches (Fig. 1). The oven was constructed from 1 inch pine wood and the upper pyramidal portion was lined with galvanized sheet metal to reflect the heat and to protect the wood. One side of the box is hinged at its junction with the pyramidal top so that it serves as a door, forming an opening into the oven which is approximately 26 by 7 inches.

The oven has no fixed bottom and when in use it is merely placed upon a piece of sheet tin on the table. A porcelain tray, $22\frac{1}{2}$ by $12\frac{1}{2}$ inches, serves as a container for the infiltrating work. This tray readily slides in and out of the oven and greatly simplifies embedding.

Heat is supplied by three 100 watt bulbs set in sockets at the top of the pyramid, and is controlled by a thermoregulator of simple construction. The thermostat consists of a piece of ordinary glass tubing, 30 cm. long and 5 mm. in diameter, on the end of which is blown a ball about 20 mm. in diameter. Approximately 50 mm. from the ball the tubing was bent to form a "U" tube, with a ball on one end and an opening on the other (Fig. 2). The tube was filled with mercury and then inverted. This procedure was repeated until the entire tube, except about 10 mm. at the open end, was filled. Then several drops of chloroform were added. The chloroform with a small air bubble was forced into the ball by inverting the tube. Following this, a sufficient amount of mercury was removed from the tube to lower the mercury level to about

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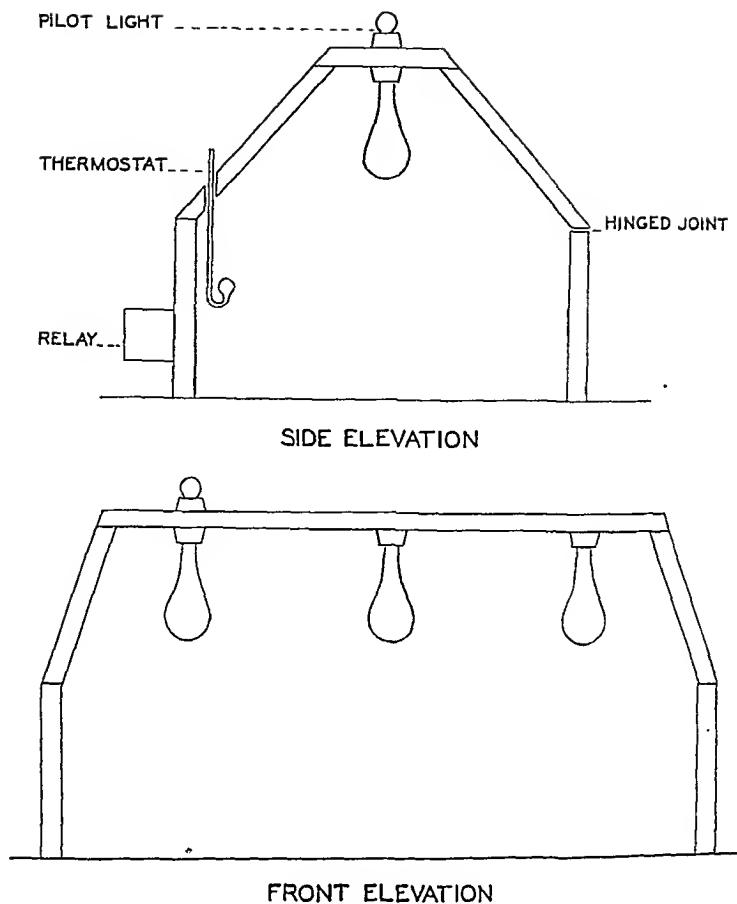


Fig. 1.—Front and side elevations showing the construction of the oven and the position of bulbs, thermostat, and relay.

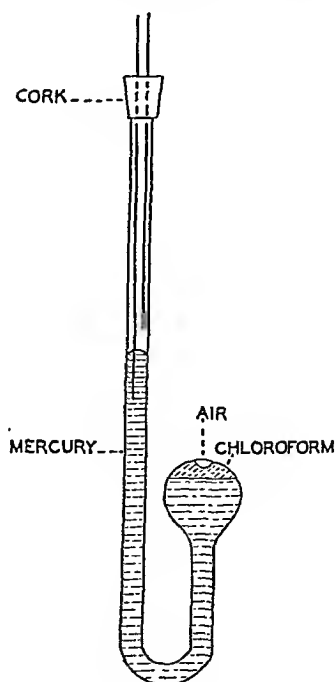


Fig. 2.—A diagrammatic representation of the thermostat showing details of construction.

100 mm. from the open end. Chloroform is added because its extreme volatility between the temperatures of 55° and 65° C. causes a large variation in the height of the mercury column and therefore greater sensitivity of the instrument. The tube is then placed in the oven with the long arm extending through a small hole in the top and is so adjusted that the ball is at the approximate level of the paraffin.

Two wires were forced through a small cork and suspended in the tube. One of the wires being longer than the other was passed below the surface of the mercury, while the other remained a few millimeters above the surface. These wires were attached to a Duoco relay which in turn regulated the current supplied to the light bulbs in the oven and to a small pilot light placed on the top of the oven. By raising or lowering the wire above the surface of the mercury, it is possible to control the temperature within the oven.

The advantage to this particular type of relay is that it operates on 110 volt current, but allows only a reduced current to pass through the thermostat, thereby eliminating the necessity of batteries or an additional transformer. Furthermore, this relay is desirable because it is so constructed as to eliminate the chatter which frequently accompanies the use of alternating current in such instruments.

The total cost involved in equipping this type of oven in our laboratories has been slightly less than \$15.00 per oven. The cost of construction of the oven, including the wiring, was \$3.90, while the relay cost \$11.00. In addition to the low cost of construction the size of the oven allows a greater amount of usable space than most ovens of the same cost which generally lack the advantages of automatic temperature control.

Several of these ovens have been in use in our pathology and histology laboratories for the past year and have been found to be very satisfactory. The time required to bring the temperature in the oven to that of the melting point of paraffin, using three 100 watt bulbs, has been found to be about twenty minutes. With the door of the oven closed, the temperature, once obtained, will remain within one degree for an indefinite period.

It is suggested that following two months of continuous use the surface of the mercury should be cleaned in order to maintain the sensitivity of the thermostat. This may be easily done with a bit of cotton on the end of a small wire.

ANTIGEN PIPETTE FOR KLINE SYPHILITIC TEST*

EDWIN S. POWELL, COLUMBIA, S. C.

A STRONG and dependable pipette for use in dropping antigen in the Kline syphilitic test may easily be made from a 1 c.c. serologic pipette, 26 gauge syringe needle, rubber medicine dropper bulb, and cement ("Dart Household and General Purpose Cement" has proved most satisfactory to me).

Select a fairly stout 1 c.c. pipette—cut off a small portion of the tip so that it will fit tight in the cup of the needle. Then cut off the top of the pipette at the 0.3 c.c. mark. By cutting down the length the pipette is easier to handle. Cut off the point of the needle about 1 mm. from the joint at the bottom of the cup by notching with a triangular file and breaking it off evenly.

Place a drop of cement on the tip of the pipette and push the needle on gently and firmly. With an applicator or match stem concentrate the cement evenly around the top of the needle so as to assure permanency and no leakage. Allow this to dry thoroughly. Put the rubber bulb on the top of pipette and it is ready for use. Should there be a loose fit between the pipette and the bulb, wrap with a rubber band.

This pipette delivers approximately 62 drops per 0.5 c.c., which is the size drop Kline recommends. Because of its speed it will be found especially useful in running large numbers of tests.

*From the Hygienic Laboratory, South Carolina State Board of Health, Columbia.
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A CONVENIENT TURNTABLE FOR STAINING JARS

A. BYRON LEONARD, PH.D., AND ALICE E. LEONARD, M.A.
LAWRENCE, KAN.

WHEN tissue sections are to be carried through an extensive series of reagents, a convenient arrangement of the containers is often difficult. The jars containing the reagents are often arranged in a long row, or in a series of shorter ones, but in any case the technician does not find all the jars within easy reach. However, by placing the staining jars on a turntable, such as the simple and inexpensive device described below, all the containers in a series become equally accessible to the operator.

Fig. 1 represents a sectional view of the device. In this illustration C is a circular piece of wood, $1\frac{1}{2}$ inches in thickness and 24 inches in diameter, which comprises the platform of the turntable. In the center of the ventral side of C a circular depression, 1 inch deep and 6 inches in diameter, is made to receive A, which is a pinion obtained from the transmission gears of an old

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automobile. The pinion *A* has a collar on its ventral side which rests within the thrust bearing *B*. The bearing *B* is held in place by being inset into the block *D*, which in turn may be secured to the top of a table. Since the bearing contains a ball race and ball bearings, the platform *C* turns very easily, even when bearing a load of Coplin jars. The cost of materials for this very convenient device was slightly more than \$1.00.

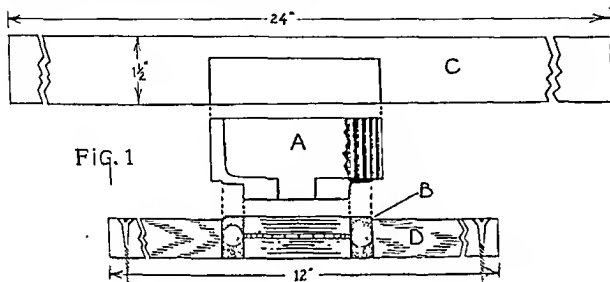


FIG. 1

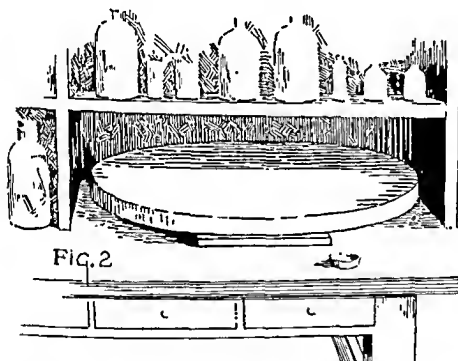


FIG. 2

Fig. 2 is a perspective view of the turntable. Upon the periphery of the platform may be placed from 20 to 22 Coplin staining jars, depending upon the size of the bases. The unoccupied space in the center of the platform makes a convenient place to put the tops of the jars when the latter are in use.

CHEMICAL

A RAPID METHOD FOR DETERMINING SPECIFIC GRAVITY OF BODY FLUIDS BY THE FALLING DROP PRINCIPLE*

CARROLL J. BELLIS, M.D., MINNEAPOLIS, MINN.

CHANGES in the density of whole blood or plasma are important in defining and recognizing the onset of shock and in determining the degree of hydremia. The well-known Hammerschlag method and its several modifications (Roy, Van Walsem, Exton) are time-consuming and elaborate. Gravimetry has the same disadvantages. Since the introduction by Barbour and Hamilton^{1,2} of the falling drop method, the determination of blood specific gravity has become an acceptable routine clinical procedure. With this technique, the blood or plasma specific gravity may be determined in one or two minutes, using only a drop of the sample (0.01 c.c.), within a maximum error of ± 0.0001 sp. gr. Guthrie⁴ and Kagan⁵ have employed the falling drop principle with an accuracy far greater than clinical experience necessitates.

In the surgical wards of the University of Minnesota hospitals we have employed the falling drop method to determine the state of hydration of patients and the plasma protein concentration. After Moore and Van Slyke⁶ had formulated an expression whereby the protein concentration could be calculated if the plasma specific gravity were known, Nugent and Towle⁷ discovered that the albumin-globulin ratio had no effect on specific gravity, since albumin and globulin exerted the same effect. Weech, Reeves, and Goettsch¹² then published formulas for accurately calculating the serum or plasma protein concentration from the specific gravity. Peters and Van Slyke⁸ have indicated that of the methods for determining serum protein, the specific gravity method is sufficiently accurate. Spencer,¹⁰ after making many comparative determinations, concluded that the falling drop method was more accurate than the weight method for determining serum protein. Similarly, the later work of Shuman and Jeghers⁹ showed that detailed chemical procedures are no more accurate for routine work than the falling drop method. This communication presents a method of simplifying and accelerating the determination of blood or plasma specific gravity with the Barbour and Hamilton technique.

METHOD

From the Barbour and Hamilton alignment chart, a logarithmic progression was derived, and from an expression which designates the points on their chart, Table I was obtained. Much time is saved by using this table to obtain the apparent density differences.

*From the Department of Surgery, University of Minnesota Medical School, Minneapolis.
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TABLE I

APPARENT DENSITY DIFFERENCES FOR VARIOUS DEGREES CENTIGRADE AND FALLING TIMES

TIME (SEC.)	20°	21°	22°	23°	24°	25°	26°	27°	28°	29°	30°	31°	32°	33°	34°
10	036	037	038	038	039	040	040	041	042	042	043	043	044	044	045
11	032	032	033	033	034	035	035	036	037	037	038	038	040	040	041
12	028	029	029	030	030	031	032	033	034	034	034	035	036	036	037
13	025	026	027	027	028	028	029	030	031	031	032	032	033	033	034
14	023	023	024	024	025	026	026	027	028	028	029	030	031	031	032
15	021	021	022	022	023	024	024	025	026	026	027	028	029	029	029
16	019	020	020	021	021	022	022	023	025	025	025	026	027	027	028
17	018	018	019	019	020	021	021	022	023	023	024	024	025	026	027
18	016	017	017	018	019	019	020	021	022	022	023	023	024	024	025
19	015	015	016	017	018	018	019	020	021	021	022	022	023	023	024
20	014	014	015	016	017	017	018	019	020	020	021	021	022	023	023
21	013	014	014	015	016	016	017	018	019	019	020	020	021	022	022
22	012	013	013	014	015	016	016	017	018	018	019	020	020	021	022
23	011	012	013	013	014	015	015	017	017	018	018	019	020	020	021
24	010	011	012	013	013	015	015	016	017	017	018	019	020	020	021
25	010	011	012	012	013	014	014	016	016	016	017	018	019	019	020
26	010	010	011	012	012	013	014	015	016	016	017	017	018	019	019
27	009	010	010	011	012	013	013	014	015	015	016	017	018	018	019
28	009	009	010	011	011	012	013	014	015	015	016	016	017	018	019
29	008	009	010	010	011	012	012	014	014	015	015	016	017	018	018
30	008	008	009	010	011	011	012	013	014	014	015	016	016	017	018
31	007	008	009	010	010	011	012	013	013	014	015	015	016	017	018
32	007	008	009	009	010	011	011	013	013	014	014	015	016	017	017
33	007	008	008	009	010	010	011	012	013	013	014	015	016	016	017
34	007	007	008	009	009	010	011	012	012	013	014	015	015	016	017
35	006	007	008	008	009	010	011	012	012	013	014	014	015	016	017
36	006	007	007	008	009	010	011	012	012	013	013	014	015	016	016
37	006	007	007	008	009	010	011	012	012	013	013	014	015	016	016
38	000	006	007	008	009	009	010	011	012	012	013	014	015	015	016
39	005	000	007	008	008	009	010	011	011	012	013	014	014	015	010
40	003	006	007	007	008	009	010	011	011	012	013	013	014	015	016
41	005	006	007	007	008	009	010	011	011	012	013	013	014	015	016
42	005	006	000	007	008	009	009	010	011	012	012	013	014	015	015
43	005	005	006	007	008	009	009	010	011	012	012	013	014	015	015
44	005	005	006	007	008	008	009	010	011	011	012	013	014	014	015
45	004	005	006	007	007	008	009	010	010	011	012	013	013	014	015
46	004	005	006	006	007	008	009	010	010	011	012	013	013	014	015
47	004	005	006	006	007	008	009	010	010	011	012	013	013	014	015
48	004	005	006	006	007	008	009	010	010	011	012	012	013	014	015
49	004	005	006	006	007	008	008	009	010	011	012	012	013	014	015
50	004	005	005	006	007	008	008	009	010	011	011	012	013	014	014
51	004	005	005	006	007	007	008	009	010	011	011	012	013	014	014
52	004	004	005	006	007	007	008	009	010	010	011	012	013	014	014
53	004	004	005	006	007	007	008	009	010	010	011	012	013	013	014
54	003	004	005	006	006	007	008	009	010	010	011	012	013	013	014
55	003	004	005	006	006	007	008	009	009	010	011	012	012	013	014
56	003	004	005	006	006	007	008	009	009	010	011	012	012	013	014
57	003	004	005	005	006	007	008	008	009	010	011	012	012	013	014
58	003	004	005	005	006	007	008	008	009	010	011	011	012	013	014
59	003	004	005	005	006	007	008	008	009	010	011	011	012	013	014
60	003	004	005	005	006	007	008	008	009	010	011	011	012	013	014
61	003	004	004	005	006	007	007	008	009	010	011	011	012	013	014
62	003	004	004	005	006	007	007	008	009	010	010	011	012	013	014
63	003	004	004	005	006	007	007	008	009	010	010	011	012	013	014
64	003	004	004	005	006	007	007	008	009	010	010	011	012	013	014
65	003	003	004	005	006	006	007	008	009	010	010	011	012	013	013
66	003	003	004	005	006	006	007	008	009	009	010	011	012	013	013
67	003	003	004	005	006	006	007	008	009	009	010	011	012	013	013
68	003	003	004	005	006	006	007	008	009	009	010	011	012	012	013
69	002	003	004	005	006	006	007	008	009	009	010	011	012	012	013
70	002	003	004	005	005	006	007	008	009	009	010	011	012	012	013
	002	003	004	005	005	006	007	008	008	009	010	011	012	012	013

For most samples of plasma we use the 75.3 : 24.7 :: m-xylene : bromobenzene mixture which these investigators suggested. For more hydremic or hypoproteinemic plasma the 76.9 : 23.1 mixture is used; it has a specific gravity of 1.013. If the specific gravity of whole blood is to be determined, the 72.1 : 27.9 mixture is used; this mixture has a specific gravity of 1.043. As will be recalled, the falling times of exactly 0.01 c.c. of standard solution of potassium sulfate and sample through 30 cm. of m-xylene-bromobenzene mixture are determined with the stop watch, and the room temperature is noted. Table I gives the apparent density difference of each falling drop, the apparent density difference of the standard solution being given a - sign, that of the sample a + sign. The algebraic sum of these two (actual density difference) is then algebraically added to the specific gravity of the standard solution to obtain the specific gravity of the sample. Table II (made from the formula of Weech, Reeves, and Goettsch) gives the protein concentration of the serum corresponding to the specific gravities. If plasma is used, reference to the same table may be made for clinical purposes, since the error thus produced is less than 0.5 Gm. per 100 c.c.

TABLE II
SERUM PROTEIN CONCENTRATIONS CORRESPONDING TO SPECIFIC GRAVITY

SP. GR.	PROTEIN	SP. GR.	PROTEIN	SP. GR.	PROTEIN
1.017	3.37	1.025	6.16	1.033	8.94
1.018	3.72	1.026	6.51	1.034	9.29
1.019	4.07	1.027	6.85	1.035	9.64
1.020	4.42	1.028	7.20	1.036	9.98
1.021	4.77	1.029	7.55	1.037	10.33
1.022	5.11	1.030	7.90	1.038	10.68
1.023	5.46	1.031	8.25	1.039	11.03
1.024	5.81	1.032	8.59	1.040	11.38

Example:

Temperature 22° C.

Falling time of serum = 30 sec.

Apparent density difference +0.009 (Table I).

Falling time of standard (sp. gr. 1.035) = 20 sec.

Apparent density difference -0.015 (Table I).

0.009 - 0.015 = -0.006 (actual density difference).

1.035 - 0.006 = 1.029 = sp. gr. of serum.

1.029 is equivalent to protein concentration 7.55 per cent (Table II).

COMMENT

To date, the improvement described, namely, the already calculated chart of apparent density differences, has made possible the determination of blood or serum specific gravity in a few moments. The constant checks of this method of obtaining plasma or serum protein concentration with micro-Kjeldahl readings have been both gratifying and reassuring, and it is my feeling that this method of estimating total plasma protein concentration is fully as accurate as the well-known Kjeldahl method.

The fluid requirement of the surgical patient has ceased to be an arbitrary value or a matter of "hit or miss." Closely correlated with the sodium chloride

intake and excretion is the water balance, and an index of this may be obtained by plasma density determinations. Thompson, Ravdin, and Frank¹¹ have significantly commented on the effects of hypoproteinemia on wound disruption, a relation which may be due to edema or to the general lack of essential elements which accompany low protein intake. Preoperative preparation of patients for extensive surgical procedures can be assisted by rapid protein determinations with this method. During the critical postoperative period similar determinations will indicate the general state of the patient.

SUMMARY

A successful experience with the Barbour and Hamilton falling drop method for determination of plasma, serum, or whole blood specific gravity is reported. A table is presented for use in the rapid determination of the specific gravity, and a second table is given for obtaining the serum protein concentration when the specific gravity is known. The advantages of these are mentioned, and some indications for their employment are cited.

REFERENCES

1. Barbour, H. G., and Hamilton, W. F.: Blood Specific Gravity: Its Significance and a New Method for Its Determination, *Am. J. Physiol.* 69: 654, 1924.
2. Barbour, H. G., and Hamilton, W. F.: The Falling Drop Method for Determining Specific Gravity, *J. Biol. Chem.* 69: 625, 1926.
3. Barbour, H. G., and Hamilton, W. F.: The Falling Drop Method for Determining Specific Gravity. Some Clinical Applications, *J. A. M. A.* 88: 91, 1927.
4. Guthrie, C. C.: An Apparatus for Quickly Measuring the Specific Gravity of Body Fluids, *J. Lab. & Clin. Med.* 17: 1158, 1932.
5. Kagan, B. M.: A Simple Method for the Estimation of Total Protein Content of Plasma and Serum. I. A Falling Drop Method for the Determination of Specific Gravity, *J. Clin. Investigation* 17: 369, 1938.
II. The Estimation of Total Protein Content of Human Plasma and Serum by the Use of the Falling Drop Method, *J. Clin. Investigation* 17: 373, 1938.
6. Moore, N. S., and Van Slyke, D. D.: The Relationships Between Plasma Specific Gravity, Plasma Protein Content, and Edema in Nephritis, *J. Clin. Investigation* 8: 337, 1930.
7. Nugent, R. L., and Towle, L. W.: The Specific Gravity of Synthetic Solutions of Serum Albumin and Serum Globulin, *J. Biol. Chem.* 104: 395, 1934.
8. Peters, J. P., and Van Slyke, D. D.: Quantitative Clinical Chemistry, Vol. II. Methods, Baltimore, 1932, Williams & Wilkins Co.
9. Shuman, H. H., and Jeghers, H.: The Value of Routine Blood-Protein Determinations, *New England J. Med.* 222: 335, 1940.
10. Spencer, H.: The Water Content of Blood Serum. A Comparison of the Determination of the Specific Gravity by the Falling Drop Method and by Several Other Methods, *Am. J. Dis. Child.* 37: 546, 1929.
11. Thompson, W. D., Ravdin, I. S., and Frank, I. L.: Effects of Hypoproteinemia on Wound Disruption, *Arch. Surg.* 36: 500, 1938.
12. Weech, A. A., Reeves, E. B., and Goettsch, E.: The Relationship Between Specific Gravity and Protein Content in Plasma, Serum, and Transudate From Dogs, *J. Biol. Chem.* 113: 167, 1935.

A SIMPLIFIED SYSTEM OF BUFFERS AND INDICATORS WITH OR WITHOUT GLASS ELECTRODES*

J. F. McCLENDON, PHILADELPHIA, PA.

BECAUSE a multiplicity of technical methods are demanded in medical laboratories, there is a need for saving the time required for mathematical calculations. For this reason, I built a potentiometer reading directly in pH.⁶ A newer development is the direct reading glass electrode apparatus that eliminates all calculation in determining pH; however, it must be standardized frequently by a standard buffer because, if the proper buffer is not used, all the results will be incorrect. The following method eliminates all complicated mathematics and all errors caused by moisture in "dry" chemicals and by inaccurate volumetric apparatus used in the preparation of standard buffers. Furthermore, with a burette of uniform bore, buffers of graded series may be prepared for determining pH with indicators.

With the realization that Sørensen's values of pH were incorrect, physical chemists eliminated this criticism by coining the term "activity." Now pH is redefined as the logarithm of the reciprocal of hydrogen-ion activity. If one adds 0.04 to Sørensen's value, the correct result is obtained.

It has been shown that the ionic strength[†] of a solution affects the ionization of both indicators and buffers (salt error). But determinations of ionic strength may be avoided through the use of buffers and indicators that are affected very little by changes in ionic strength. Also calculations may be eliminated by the use of special graph paper.[‡]

An approximation should be made when these solutions are prepared. Otherwise difficulties may arise if solubilities or safe dilutions are exceeded. However, quantitative accuracy is not necessary except where indicated.

Indicators will act as buffers if they are at high concentration; hence, if they are to serve as indicators, they should be in very low concentration. Because of the low solubility of some indicators it has been customary to make their solutions 0.01 to 0.04 per cent. For uniformity of practice it is desirable to make the more soluble ones of the same concentration. Since dry indicators may not always wet with water and are more soluble in alcohol and since alcohol checks mold growth, it is well to weigh out 0.04 Gm. of the indicator, add 50 c.c. of alcohol to dissolve it (heat may be necessary), and then add 50 c.c. of water (some indicators will not completely dissolve until after the addition of water).

Methyl orange is very little, if at all, affected by ionic strength. Its "absorption" spectrum is given in Fig. 1 for both acid and alkaline solution. When light of wave length 5600 A. U. was used, the "absorption" (logarithm of

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†Ionic strength = $\frac{1}{2}$ (sum of concentration of each ion \times its valence squared).

‡For sale at Hahnemann Medical College Book Shop, 235 N. 15th Street, Philadelphia.

the reciprocal of the transmittance) of the acid (un-ionized*) dye was 0.62, and the absorption of the alkaline (ionized) dye was 0.05; therefore the relative amount of the alkaline (ionized) dye may be determined. At pH 3.6 the absorption was 0.335; hence 50 per cent was ionized ($0.62 + 0.05$ divided by 2

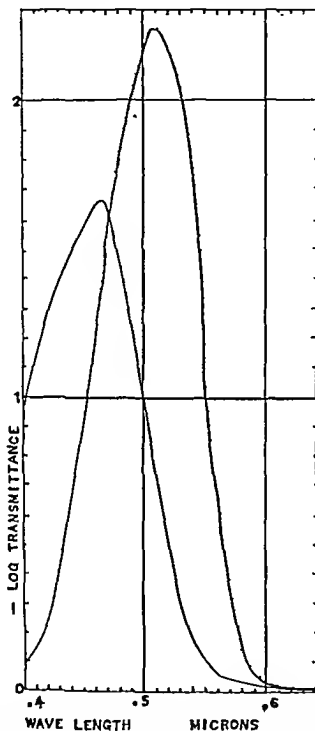


Fig. 1—Absorption curves at 25° C. of 1 c.c. of 0.04 per cent methyl orange in 25 c.c. of N/10 HCl (tall rough curve) and N/100 NaOH (short smooth curve) made with a Coleman spectrophotometer. The ionization constant was computed from the absorption at 5600 Å. U. (0.56 μ), and pH 1, 12, and 3.6 were determined with a Coleman glass electrode. The part above 2 is not graduated since this method becomes less accurate as complete absorption is approached.

equals 0.335). Fig. 2 shows on the left the amount of yellow (a) in the dye at different pH values from 1.6 to 5.6. (The red component of the dye is represented by $1 - a$ on the right.) A similar graph for phenol red† is also shown in Fig. 2, where red component is represented by a .

*It is usually represented as a "zwitterion" or amphoteric ion, but this may be considered un-ionized since the hydrogen ion that separated at one point attached itself at another.

†The salt correction for phthaleins (Kolthoff¹⁰) is related to buffer solutions of ionic strength equal to 0.1.

Ionic strength

0.50
0.05
0.01
0.005
0.0025

Correction

-0.20
+0.11
+0.13
+0.15
+0.16

Phenolphthalein behaves as a dibasic acid,* and its dissociation graph appears to be a composite of the two graphs in Fig. 2. A drop of phenolphthalein solution is used in the preparation of the salt component of the buffers, but this will not interfere with the use of other indicators at pH values less than 8. Similar graphs have been prepared for a number of other indicators.^{7-9, 11, 12}

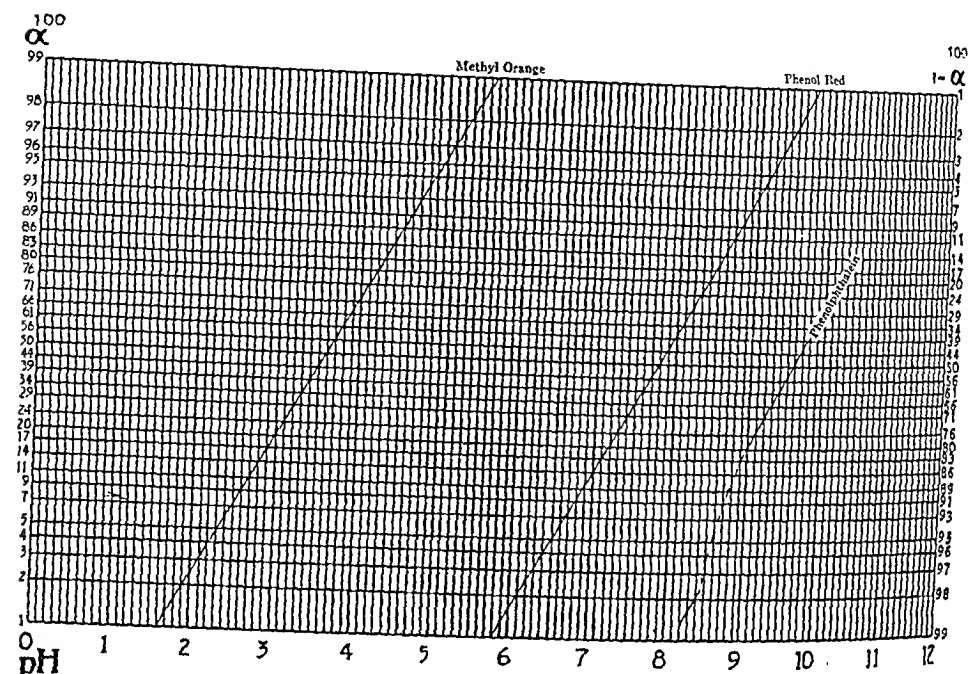


Fig. 2.—Percentage at 25° C. of methyl orange, phenol red, and phenolphthalein in the acid form (100 α , left) and alkaline form [100 (1- α), right].

STOCK SOLUTIONS

Normal hydrochloric acid: Place 10 c.c. of concentrated HCl (approximately 38 per cent by weight, 45 per cent by volume, sp. gr. 1.19) in a 100 c.c. volumetric flask and dilute to 100 c.c. Transfer to a 200 c.c. glass-stoppered bottle and add 23 c.c. of water.[†]

Carbon dioxide-free water: Place 700 c.c. of distilled water in a round bottom, liter pyrex flask. Cover with a watch glass and boil vigorously for five minutes. Insert an oversized rubber stopper and cool under tap. Allow to remain stoppered until used.

Normal sodium hydroxide: Place 75 Gm. of NaOH and 75 c.c. of water in a pyrex flask, 200 c.c. or larger. Rotate until dissolved. Transfer the hot solution to a 100 c.c. pyrex flask. Place on the surface of the liquid a lump of paraffin, which will melt and exclude air. Wrap the flask in a towel to delay cooling and let it stand until the next day when the carbonate should have settled, at least from the top layer. Lift the solidified paraffin and insert the

*Michaelis and Gyemant¹³ speak of three dissociation constants, but Kober and Marshall¹⁴ found the trisodium salt to be colorless. Rosenstein¹⁵ observed two dissociation constants.

[†]For more accurate N/10 HCl place the 1:1 acid in a still, distill off three-fourths, collect 25 c.c., and read barometer. At 760 mm. weigh out 18.017 Gm.; at 750 mm., 17.996 Gm.; at 740 mm., 17.975 Gm.; at 730 mm., 17.953 Gm.: Then dissolve and dilute to 100 c.c. (Hulett and Bonner²).

mouth end of a 10 c.c. pipette and suck up clear solution until the bulb is filled (reversal merely saves time). Run the solution into a 100 c.c. volumetric flask and fill to the mark with carbon dioxide-free water. If the flask will hold 10 c.c. more, run in 10 c.c. carbon dioxide-free water. This may be done with the same pipette, preferably without rinsing, by using a rubber tube to protect the mouth from sodium hydroxide and then sucking the solution up as far as the rubber tube to rinse the stem. Mix by inversion and rinse the pipette by sucking up several times; then transfer 10 c.c. to a small beaker or Erlenmeyer flask for titration with hydrochloric acid. Use one drop of phenolphthalein solution as indicator, and titrate to the end point, the first pink coloration that remains twenty seconds after stirring. Record the titer as 10x hydrochloric acid and add 10x carbon dioxide-free water to the 100 c.c. of sodium hydroxide on transferring to a paraffin-lined bottle. The paraffin lining is made by pouring about 20 Gm. of melted paraffin (preferably with melting point over 60° C.) into a dry 200 c.c. cylindrical, glass-stoppered bottle and rolling it on the table while cooling (having dipped the dry stopper in melted paraffin to prevent sticking with sodium hydroxide).

Normal acetic acid: Mix 6 c.c. of glacial acetic acid with 100 c.c. of water.

Normal chloroacetic acid: Weigh out 95 Gm. of monochloroacetic acid (free from acetic acid, dichlor and trichlor), and place it in a 100 c.c. volumetric flask. Add water to dissolve the acid, and fill the flask to the mark.

Normal cacodylic acid: Weigh out 13.8 Gm. of cacodylic acid, and place it in a 100 c.c. volumetric flask. Add water to dissolve the acid, and fill the flask to the mark.

CONCENTRATED BUFFERS FOR STANDARDIZING GLASS ELECTRODES

Acetic-acetate buffer: Using the same pipette so that correction for error will not be necessary, place 25 c.c. of normal acetic acid in a 100 c.c. glass-stoppered bottle and 25 c.c. in a small beaker or Erlenmeyer flask, and add one drop of phenolphthalein solution. Fill a burette with normal sodium hydroxide and run in 20 c.c.; then very carefully titrate to the first pink coloration that stays after stirring for twenty seconds. Transfer this neutralized solution (sodium acetate) to the bottle, using very small amounts of carbon dioxide-free water to rinse the titration vessel. Pour rinse water into the bottle. The pH of this 50/50 mixture of acetic acid and its sodium salt will be 4.74 at 25° C. and will remain constant on dilution up to ten times or more with carbon dioxide-free water.

Chloroacetic-chloroacetate buffer: A 50/50 mixture of chloroacetic acid and sodium chloroacetate may be made in a similar manner. The pH of this solution will be 2.86 at 25° C. (Armstrong¹ found 2.8.)

Cacodylic-cacodylate buffer: A 50/50 mixture of cacodylic acid and sodium cacodylate may be made in a similar manner except that the titration should not be stopped at a pale pink but continued to maximum color. The pH of the 50/50 mixture will be 6.2 at 25° C.

Borax buffer: A solution of borax may have a pH of 9.15 to 9.2, but its pH is influenced by the ionic strength and therefore is changed by dilution. Its advantage lies in the fact that no titration is necessary. The use of carbon

dioxide-free water in dissolving it is imperative, and its solution must be protected from the carbon dioxide of the air.

PREPARATION OF A GRADED SERIES OF BUFFERS FROM pH 2 TO 8

The titration graph of one of the acids listed above with sodium hydroxide using a glass electrode as indicator gave the clue for the preparation of buffers of any pH within its range. But it is not necessary for each investigator to make this titration graph over again. By using the special graph paper (Fig. 3) the titration graph becomes a straight line running through the pH value of the 50/50 buffer at its midpoint and a point 2 pH units to the left at the lower margin. The unit denoted by α on the left margin denotes the fraction of the acid neutralized by sodium hydroxide. Because this titration graph assumes that the free acid is undissociated, it cannot be used for any but very low concentrations of hydrogen ions, certainly not for pH less than 2.

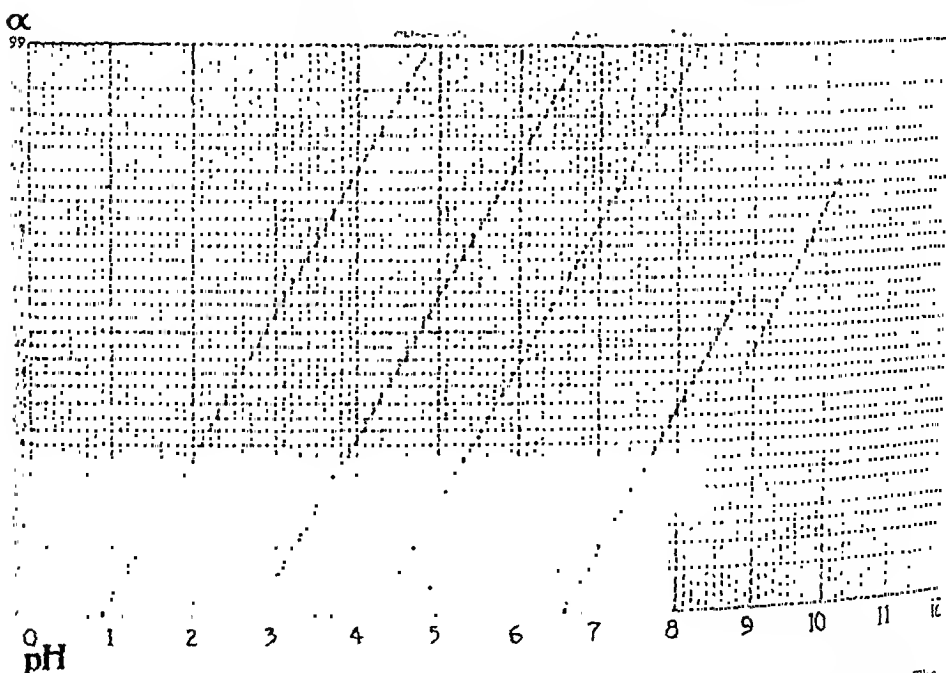


Fig. 3.—Titration graphs at 25° C. of chloroacetic, acetic, cacodylic, and boric acids. The fraction neutralized is represented by α on the left. Hitchcock and Taylor give slightly different values. The boric curve on the left assumes borax as completely, and on the right, as half neutralized.

From Fig. 3 it is seen that cacodylic acid covers the pH range from 4.2 to 8.2. In order to make a buffer of any pH within this range without calculations it is necessary for the "normality" of the normal sodium hydroxide and the cacodylic acid to be exactly equal. Therefore, pipette 10 c.c. of cacodylic acid into a titrating vessel, add one drop of phenolphthalein solution, and titrate with the sodium hydroxide to the maximum color that remains twenty seconds after stirring. Read the burette, and if 11 c.c. (for instance) have been required, add 10 per cent distilled water to the remainder of the cacodylic acid; if 9 c.c. (for instance) have been required, add 1 c.c. of water to each 9 c.c. of sodium hydroxide solution.

To prepare any buffer between pH 4.2 and 8.2 trace the pH coordinate to the cacodylic acid graph and note the α coordinate that it intercepts. If x represents the number of cubic centimeters of cacodylic acid chosen, add αx cubic centimeters of sodium hydroxide solution. Similar procedures may be made with acetic and chloroacetic acids.

Boric acid behaves in a peculiar manner (Fig. 3). If we assume boric acid to be monobasic and titrate normal boric acid with normal sodium hydroxide, a straight line from about pH 8.8 to 10.2 is obtained, with pH 9.15 representing half neutralization (as borax). But if we assume borax to be the fully neutralized "salt," then boric acid is only half monobasic, and when it is titrated with 0.5 normal sodium hydroxide, the straight line from about pH 6.5 to 8.8 is obtained. This abnormal behavior, together with the sensitivity of borate buffers to ionic strength and the rapidity with which they absorb carbon dioxide from the air, makes the use of borates undesirable.

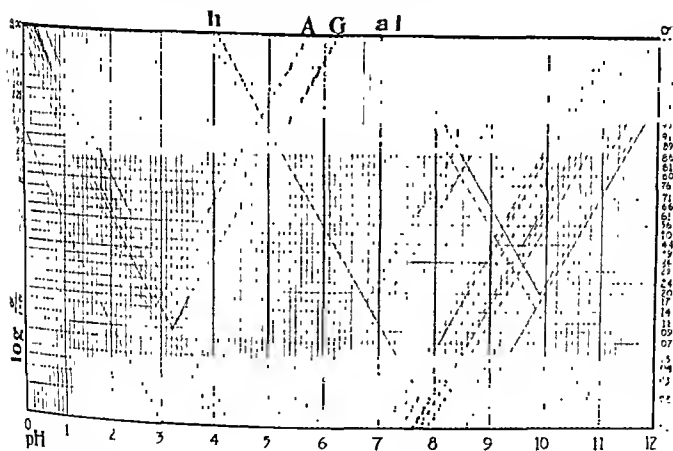


Fig. 4.—Ionization graphs at 25° C. of the amino acids of protein hydrolyzates. *A*, aspartic acid; *h*, histidine; *G* (double line), glutamic and hydroxyglutamic acids; *a*, arginine; and *l*, lysine. The ionization graphs of the amino groups slope in the opposite direction to those of the carboxyl groups.

BUFFER ACTION OF AMINO ACIDS

In attempting to buffer solutions containing protein hydrolytic products one should bear in mind that they themselves have a powerful buffer action, as shown in Fig. 4. In this chart aspartic acid is marked *A*; glutamic and hydroxyglutamic, *G*; histidine, *h*; arginine, *a*; and lysine, *l*. The dissociation graphs of the other amino acids are so close together as to make labeling difficult. On the addition of any buffer composed of partly neutralized acid, the acid would partly combine with the amino groups of these amino acids and the base would distribute itself between the carboxyl groups of these amino acids and the

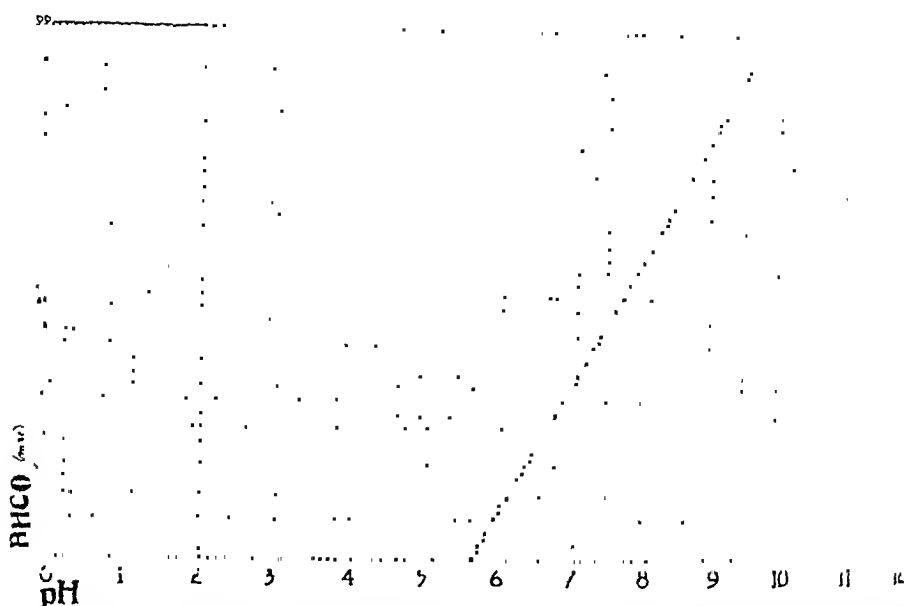


Fig. 5.—Carbonic acid-bicarbonate graph at 38° C. On the left is marked the bicarbonate on the right, the partial pressure of carbon dioxide necessary to produce the pH given at the bottom.

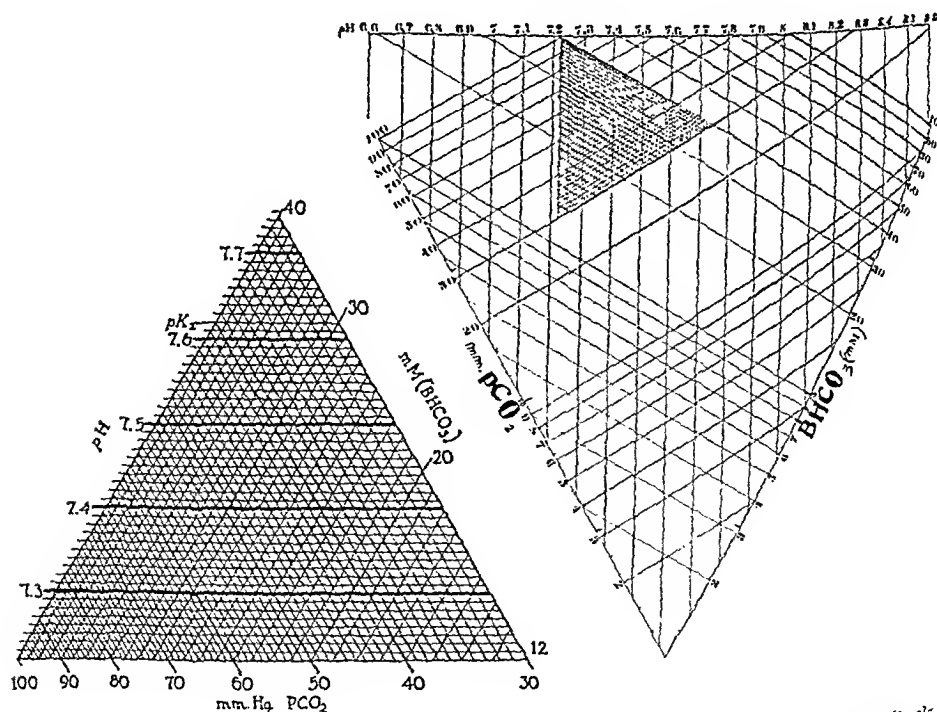


Fig. 6.—Graph of relation at 38° C. between the concentration of bicarbonate in millimoles per liter, the pressure of carbon dioxide in millimeters of mercury, and the pH. The smaller triangle on the right is enlarged on the left to emphasize the physiologic range. The graph on the right starts at 1 mm. carbon dioxide pressure: the atmospheric pressure is about 0.2 mm. Whenever Ringer's fluid, for instance, containing bicarbonate is in contact with living tissue, the carbon dioxide pressure tends to be higher than atmospheric, and perhaps the range is sufficient for ordinary laboratory conditions.

added buffer acid. It is much simpler to use the buffering power of the amino acids themselves, adjusting the pH by additions of sodium hydroxide (or hydrochloric acid) with the aid of an indicator or glass electrode. In this chart the amphoteric ions (zwitterions) are represented as undissociated molecules since they do not affect the pH although they do affect the ionic strength.

BICARBONATE BUFFERS

Just as borate buffers may change by absorption of carbon dioxide from the air, bicarbonate buffers may change by loss of carbon dioxide to the air and can be preserved only if they are kept under a fixed partial pressure of carbon dioxide. Fig. 5 is a graph at 38° C. of pH of bicarbonate solutions of concentration shown on the left margin; pressure of carbon dioxide is given on the right margin. This graph is limited, however, by the fact that there is only one pressure of carbon dioxide for each concentration of bicarbonate. To extend the range the triangular form of graph shown in Fig. 6, right, may be used with the physiologic range enlarged at the left.*

REFERENCES

1. Armstrong, W. D.: Microdetermination of Fluorine, *Indust. & Engin. Chem.* 8: 384, 1936.
2. Hulett, G. A., and Bonner, W. D.: A Method for the Preparation of Hydrochloric Acid Solutions, *J. Am. Chem. Soc.* 31: 390, 1909.
3. Hitchcock, D. I., and Taylor, A. C.: A Standardization of the pH Scale at 38°, *J. Am. Chem. Soc.* 60: 2710, 1938.
4. Kober, P. A., and Marshall, T. J.: Phenolphthalein and Its Colorless Salts, *J. Am. Chem. Soc.* 33: 59, 1911.
5. Kolthoff, I. M.: The "Salt Error" of Indicators in the Colorimetric Determination of pH, *J. Physiol. Chem.* 32: 1820, 1928.
6. McCleendon, J. F.: A Direct Reading Potentiometer for Measuring Hydrogen-Ion Concentrations, *Am. J. Physiol.* 38: 186, 1915.
7. —: Chart for the Conversion of Colorimetric Readings Into Hydrogen-Ion Concentration, *J. Biol. Chem.* 54: 647, 1922.
8. —: The Determination of Hydrogen Ions in the Gastric Contents, *J. Biol. Chem.* 59: 437, 1924.
9. —: Hydrogen Ion Determination, *Am. Naturalist* 64: 289, 1930.
10. —: On the Graphic Representation of Ionic Equilibria in Blood Serum, *Science* 81: 569, 1935.
11. —, and Medes, G.: *Physical Chemistry in Biology and Medicine*, Philadelphia, 1925, W. B. Saunders Co., pp. 110-112.
12. —, Russell, S., and Tracy, E.: The Determination of Hydrogen Ions in the Blood With the Aid of the Duboscq Colorimeter and Orthochrome-T or Paranitrophenol, *J. Biol. Chem.* 70: 705, 1926.
13. Michaelis, L., and Gyemant, A.: Die Bestimmung der Wasserstoffzahl durch Indicatoren, *Biochem. Ztschr.* 109: 165, 1920.
14. Rosenstein, L.: Ionization Constant of Phenolphthalein, *J. Am. Chem. Soc.* 34: 1117, 1912.

*McCleendon (1935). This triangular graph paper may be purchased at Hahnemann Medical College, 235 N. 15th Street, Philadelphia.

THE MEASUREMENT OF THE CHLOROFORM-SOLUBLE FRACTION OF BILIRUBIN IN PERSONS WITH JAUNDICE AND ITS SIGNIFICANCE*

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IT IS well known that the bilirubin in serum or plasma of patients with obstructive or hepatogenous jaundice gives a different reaction with diazotizing (van den Bergh) reagents from that given by bilirubin in specimens from persons with acholuric jaundice.¹ This can be demonstrated by noting the speed with which a color appears after the reagents and serum are mixed. It has been postulated that the difference in the reaction is due to two different types of bilirubin, but the underlying chemical or physicochemical causes of the difference have not as yet been definitely determined. That both types of pigment are present in some cases of jaundice has been suggested by some investigators.² In a recent paper we showed that an interpretation of the so-called biphasic van den Bergh reaction obtained when both pigments are present is often very difficult.³ It seemed desirable, therefore, to investigate another method of distinguishing between the two forms of bilirubin.

Many years ago Grunenberg⁴ observed that the bilirubin was wholly soluble in chloroform in persons who gave an indirect, slow van den Bergh reaction. Since this report attempts at the separation of two types of bilirubin in the serum by means of chloroform have been made by Varela and his co-workers,^{5, 6} and more recently by Greene and associates.⁷ The former workers studied various types of jaundice, especially the hepatogenous and the obstructive groups, and commented upon the different amounts of the chloroform-soluble fraction. In their analyses the chloroform-soluble portion was concentrated by evaporation after extraction, redissolved in alcohol, and diazotized. The residue which contained the water-soluble or direct-reacting portion was also diazotized, and the sum of the two fractions then gave the total amount of bilirubin in the serum.

In trying to repeat this work according to their technique we encountered certain difficulties. The diazotization of the two fractions did not always yield colors that could be easily compared with that of the cobalt standard. This same difficulty has been commented upon by many workers who have used the van den Bergh reaction, and various modifications of the test for avoiding the difficulty have been proposed. We tried several of these but found that they were not completely satisfactory. Our preliminary experiments showed that slightly acidified solutions of pure bilirubin in chloroform could be accurately quantitated by comparison with the 1:6,000 water solution of potassium di-

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chromate standard used by Ernst and Förster.⁸ Since this method did not involve diazotization, the difficulty mentioned was avoided.

When the yellow chloroform-soluble pigment was concentrated according to the technique of Varela and his co-workers, and redissolved in chloroform and alcohol to a definite volume, it was found on comparison with the dichromate standard, both before and after concentration, that there was a loss of approximately 50 per cent of the yellow color. Because of this it was decided not to concentrate the extract but to use a direct comparison between the yellow color given by the chloroform-soluble pigment and the dichromate standard. The accuracy of this determination was checked in two ways: first, by measurement of the color of known amounts of bilirubin dissolved in chloroform as previously mentioned; second, by demonstrating the quantitative recovery of injected bilirubin. For this bilirubin was injected into normal persons or those with hypochromic anemia. Samples of blood were taken both before and within three to five minutes after the injection. The quantity of bilirubin found in the chloroform extract of the sample after injection compared closely with the total increase of the bilirubinemia as estimated by the quantitative van den Bergh test. When such samples were tested with the qualitative van den Bergh reaction, it was found that the reaction was always of the indirect or delayed type. The results of these experiments have been described in a previous paper.³

The study of a group of patients with increased bilirubinemia due to various causes selected from the services of the Buffalo General Hospital is presented here. All specimens of sera or plasma were subjected to chloroform extraction, and the total amount of the chloroform-soluble bilirubin fraction was estimated. The bilirubin in a second portion of the serum or plasma was determined quantitatively by the Thannhauser and Andersen method.⁹ The relationship between the quantity of the chloroform-soluble or indirect-reacting bilirubin and the total amount were charted in an attempt to see whether there were any correlations between the various types of jaundice, and whether the obstructive or hepatogenous groups could be separated from one another. The latter, from a clinical point of view, would be very desirable.

The technique for the estimation of the chloroform-soluble fraction, which can be employed even in the presence of a moderate degree of hemolysis, is as follows: 1 c.c. of the serum or plasma is placed in a centrifuge tube. Three to 4 c.c. of chemically pure chloroform is then added. The tube is capped with the thumb and shaken vigorously for twenty to thirty seconds. It is then centrifuged for two to three minutes. The lower yellow chloroform layer is transferred to a graduated 15 c.c. centrifuge tube. This is done with a fine-tipped pipette inserted through the upper thick layer down to the bottom of the tube. Extraction is repeated until the chloroform remains colorless. Each successive fraction is added to the original in the graduated tube. Usually two or three extractions will remove all of the soluble pigment; occasionally, five or six are necessary. The total amount of chloroform-soluble bilirubin is now estimated in the following manner: The total quantity of the pooled fractions in the graduated tube is noted. Turbidity is rarely present, but when it is it can be cleared by placing the tube in a warm water bath for a few seconds. A small portion of this is now removed, and its color is compared

with that of the 1:6,000 potassium dichromate solution in a microcolorimeter with cups of a capacity of 1 to 2 c.c. The following calculation was derived from our experiments, and is the same as that which Soffer¹⁰ used in the bilirubin excretion test in which acetone is employed as the solvent:

$$\text{Total amount of CHCL}_3 \times \frac{\text{Reading of standard}}{\text{Reading of unknown}} \times 0.329 = \text{Milligrams of bilirubin per 100 c.c.}$$

The patients studied have been arbitrarily divided into the following groups: (1) hepatogenous jaundice, which included so-called catarrhal jaundice, acute yellow atrophy, portal cirrhosis, toxic hepatitis due to arsphenamine, toxic hepatitis accompanying overwhelming bacterial sepsis; (2) obstructive jaundice due to common duct stone, carcinoma of the head of the pancreas, obstructive biliary cirrhosis; (3) mild jaundice accompanying chronic passive congestion; (4) hemolytic jaundice, including congenital acholuric jaundice, pernicious anemia and allied states, mild jaundice accompanying malarial therapy for syphilis of the central nervous system; (5) two patients who fall in the group described by Rozendaal, Comfort, and Snell¹¹ as functional jaundice which gives the indirect van den Bergh reaction. Some persons in most groups were studied by successive determinations through the course of the disease.

TABLE I

	GROUP	NUMBER OF DETERMINATIONS	AVERAGE VALUE OF TOTAL QUANTITY OF CHLOROFORM-SOLUBLE BILIRUBIN (MG./100 C.C.)	AVERAGE PERCENTAGE OF CHLOROFORM-SOLUBLE PIGMENTS TO THE TOTAL QUANTITATIVE VAN DEN BERGH TEST (PER CENT)
I	Hepatogenous	74	0.89	24.0
II	Obstructive	38	0.68	27.9
III	Chronic passive congestion	54	0.64	61.0
IV	Hemolytic	34	1.07	74.0
V	Functional	4	1.11	86.7

The average values of the chloroform-soluble portion and the average percentage of the total amount of bilirubinemia which it forms have been estimated for the various groups and recorded in Table I. In addition, the individual values for each specimen studied have been charted according to their frequencies. One chart records the total quantity of the chloroform-soluble fraction and the other records the percentage which it forms of the total amount of bilirubin as estimated by the quantitative van den Bergh reaction. Each dot represents a single determination.

COMMENT

It will be noted from Table I that there is little difference between the average values for the patients grouped under "hepatogenous" and "obstructive" jaundice. This is true both for the chloroform-soluble fraction and for the percentage which it forms of the total bilirubin. Any attempt to separate these two clinical conditions on the basis of these average values would not

seem to be justified. Furthermore, many persons in both groups with a high degree of bilirubinemia had relatively small amounts of chloroform-soluble bilirubin when compared with the total amount present. Nevertheless, even though this amount was a small proportion of the total, it was considerably greater than that found as a result of normal red blood cell destruction. If one considers the usual amount of bilirubin in the blood stream a result of the balance between normal red blood cell destruction and the ability of the liver to remove it, then the accumulation of it in the blood stream in the absence of increased red blood cell destruction should be some indication of the functional state of the liver. If this is true, then the measurement of the chloroform-soluble or indirect-reacting pigment in cases of severe jaundice may be regarded as an estimation of liver function. In essence, this would seem to be the same measure of function as that utilized in von Bergmann's¹² bilirubin excretion test, but this proposed chloroform method can be applied when jaundice is present.

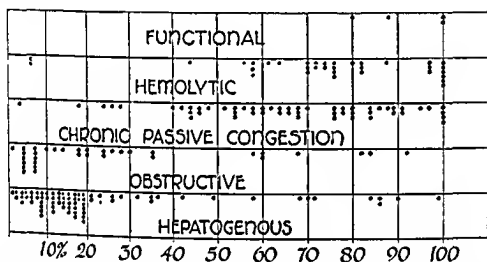


Chart L.—Percentage which the chloroform-soluble bilirubin forms of the total quantity of bilirubin as estimated by the quantitative van den Bergh reaction. Each dot represents a single determination.

Some persons had values of chloroform-soluble pigment, ranging from 1.5 to 3.5 mg. per 100 c.c., which amounted to only 15 to 30 per cent of the total amount of bilirubinemia. However, this quantity of pigment is three to six times the amount normally found, and, in our opinion, indicates an impairment of the ability of the liver to perform its normal excretory function. Furthermore, the frequency charts show that greater numbers of the higher values of the chloroform fraction occur in the hepatogenous group. It would seem reasonable that this should be so because it is in this group that the pathologic process involves the liver cells diffusely, and one should expect that the function of excreting the bilirubin derived from the breakdown of red blood cells would be impaired to a greater degree in this group than in obstructive jaundice where the primary process does not involve the liver cells directly. We feel that this confirms our belief.

That both fractions are present in persons with severe jaundice of this nature has been commented upon only recently.¹³ That their presence was not recognized earlier when the qualitative van den Bergh test was done in the usual way resulted from the fact that the immediacy of the reaction is so marked in the presence of a relatively large amount of direct-reacting pigment, that the slowly progressive reaction of the indirect pigment is obscured.³

In the groups of persons with chronic passive congestion it is seen that the greatest portion of the total amount of bilirubin is chloroform soluble. In some cases the pigment was wholly of this type, and this was usually so when the congestion was at its height. Again it would seem reasonable to consider that the function of a markedly congested liver is impaired. It may be argued that some of the bilirubinemia resulted from the breakdown of infarcts. However, in two persons with rather high values subsequent autopsy showed no evidence of infarction, either recent or old. This increase of the chloroform-soluble pigment is also in accord with the fact that most of the cases give an indirect or biphasic van den Bergh reaction. We studied this latter reaction and found it to be the result of the combination of a relatively large amount of indirect-reacting pigment with a small amount of direct-reacting pigment.³

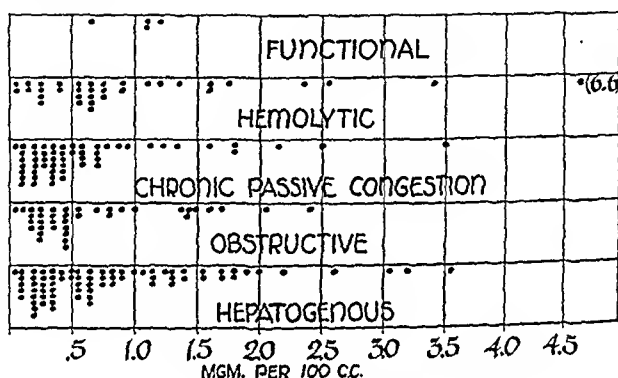


Chart 2.—Total quantity of chloroform-soluble bilirubin in each specimen. Each dot represents a single determination.

Those persons whose jaundice was due to excessive hemolysis were found to have the greater portion of the total amount of bilirubin in the chloroform-soluble form as seen in Table I and Charts 1 and 2. This coincides with the frequent observation that they give a delayed or indirect van den Bergh reaction. However, if the concentration of the total pigment is relatively high, a prompt color development may be noted upon addition of the diazotizing reagent, but this is always of the slowly progressive type, requiring fifteen to thirty minutes to reach its maximum intensity.

The cases in the last group of functional disability of the liver show the greatest portion of the pigment to be soluble in chloroform. This also conforms with the observed indirect van den Bergh reaction.

CONCLUSIONS

1. A convenient and simple method for determining quantitatively the solubility of bilirubin in chloroform has been described.
2. This method is applicable to the measuring of the so-called indirect or delayed-reacting pigment in persons with jaundice.
3. It is suggested that a measurement of this portion may serve as an index of the potential capacity of the liver when jaundice is present and when excessive hemolysis is absent.

REFERENCES

1. van den Bergh, A. A. Hijmans, and Muller, P.: Über eine direkte und eine indirekte Diazoreaktion auf Bilirubin, *Biochem. Ztschr.* 77: 90, 1916.
2. Fiegl, J., and Querner, E.: Bilirubinämie in ihren physiologisch-chemischen Beziehungen mit besonderer Berücksichtigung der diagnostischen Bedeutung, *Ztschr. f. d. ges. exper. Med.* 9: 153, 1919.
3. Heilbrun, N., and Hubbard, R. S.: An Experimental Study of the Biphasic van den Bergh Reaction, *Am. J. Clin. Path.* 8: 273, 1938.
4. Grunenberg, K.: Ueber die Topik der Umwandlungsstadien der Chloroformlöslichkeit des Bilirubins, *Ztschr. f. d. ges. exper. Med.* 35: 128, 1923.
5. Varela, B., Recarte, P., and Esculies, J.: Méthode simplifiée pour la séparation et le dosage isolé des deux bilirubines, directe et indirecte, du sérum sanguin dans les icteres, *Compt. rend. Soc. de biol.* 108: 1009, 1931.
6. Varela-Fuentes, B., and Vianna, C.: Les bilirubines, directe et indirecte, du sérum dans les icteres chroniques. Valeurs données par leur dosage séparé, *Compt. rend. Soc. de biol.* 116: 1187, 1934.
7. Greene, C. H., Plotz, M., and Localio, S. A.: Liver and Biliary Tract; Review for 1937, *Arch. Int. Med.* 61: 655, 1938.
8. Ernst, Z., and Förster, K.: Über die Bestimmung des Blutbilirubins, *Klin. Wchschr.* 3: 2386, 1924.
9. Thannhauser, J. S., and Andersen, E.: Methodik der quantitativen Bilirubinbestimmung in menschlichen Serum, *Deutsches Arch. f. klin. Med.* 137: 179, 1921.
10. Sofer, L. J.: Bilirubin Excretion as a Test for Liver Function During Normal Pregnancy, *Bull. Johns Hopkins Hosp.* 52: 365, 1933.
11. Rozendaal, H. M., Comfort, M. W., and Snell, A. M.: Slight and Latent Jaundice; Significance of Elevated Concentrations of Bilirubin Giving Indirect van den Bergh Reaction, *J. A. M. A.* 104: 374, 1935.
12. von Bergmann, G.: Zur Funktionellen Pathologie der Leber, insbesondere der Alkohol-Ätiologie der Cirrhose, *Klin. Wchschr.* 6: 776, 1927.
13. Snell, A. M., and Magath, T. B.: Use and Interpretation of Tests for Liver Function; clinical review, *J. A. M. A.* 110: 167, 1938.

A SIMPLIFIED CONTINUOUS EXTRACTOR FOR ESTROGENS AND ANDROGENS*

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THOROUGHNESS in the extraction of urine being the first essential for the proper assay of a given specimen, we have developed a continuous extractor which embodies simplicity with economy without sacrificing efficiency. The extractor was designed on the basic principle of that employed by Koch¹ in his much larger extractor. It consists of an all-glass apparatus made up of five units; a round bottom flask with a $\frac{40}{35}$ joint which can be made interchangeable with flasks of various sizes, an Erlenmeyer liter flask of heavy glass suitable for vacuum work, an ordinary Hopkins condenser, a side arm and upright portion connecting the first three mentioned units, and a filter tube containing a sintered glass bottom. The various measurements of the apparatus† are given in Fig. 1. The extractor can be used singly or in a battery of as many units as is desired. We found that a battery of four extractors makes a

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†This unit can be secured from the Scientific Glass Co., Bloomfield, N. J.

practical set-up and that if necessary multiple batteries of four each can be placed in position, each battery facing another. To facilitate working with a battery, an extractor rack was designed from $\frac{3}{8}$ inch gas pipe to which a vacuum line, water line, and overflow line for the water were permanently attached. Each unit was connected separately to its own three lines, and the vacuum was connected with a common vacuum pump. An adjustable bar which can be raised or lowered holds the clamp that fastens the round bottom flask securely. A battery of four extractors occupies a space 26 inches in width and only 22 inches in depth, including the space occupied by the electric hot plate,* which is long enough for four flasks. Figs. 2 and 3 give the details of design and show the compactness of the battery.

CONTINUOUS EXTRACTOR

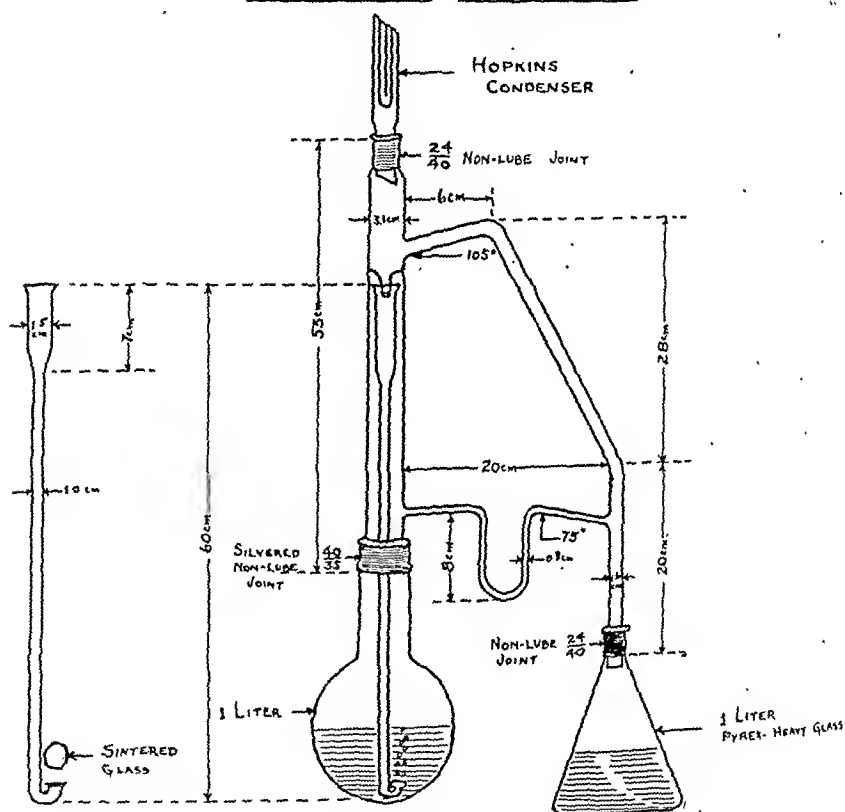


Fig. 1.—Details of construction extractor.

The methods employed in our laboratories are modifications of generally accepted methods.^{2,3} The apparatus can be used for the extraction of both estrogens and androgens. Seven hundred and fifty cubic centimeters of previously hydrolyzed urine are placed in the round bottom flask, and a like amount of benzene is introduced into the Erlenmeyer flask which is placed on the hot plate. By using a vacuum we were able to speed up the volatilization of the benzene and thus hasten the extraction. The side arms and the inner filter tube are arranged so that all of the volatilized and condensed benzene must pass through the urine that is being extracted. The incline on the distal arm of the

trap aids siphoning of the condensed benzene back into the Erlenmeyer flask and a continuous process proceeds. The sintered glass on the filter tube acts as a diffusing medium so that a maximum amount of contact between the benzene and urine ensues.

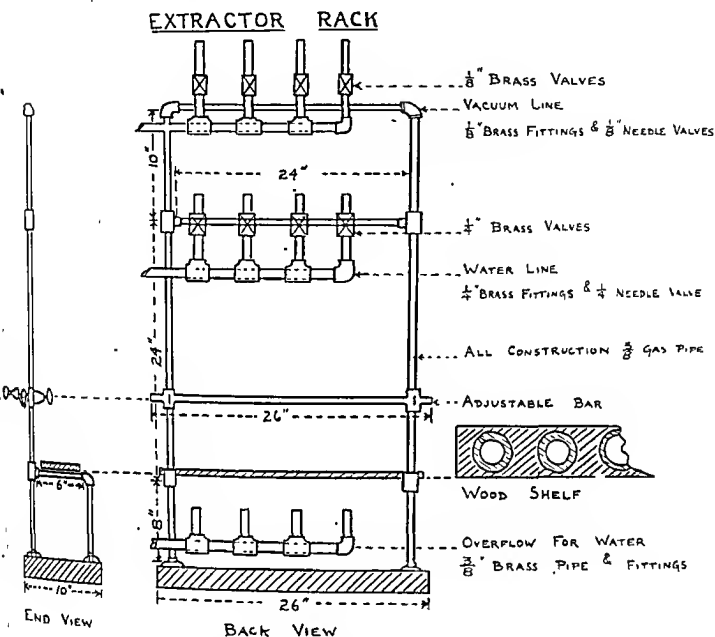


Fig. 2.—Details of construction of extractor rack.

It has been suggested⁴ that approximately twelve volumes of benzene should be passed through the urine to be extracted in order to secure the maximum yield. It has further been observed that for androgenic extraction a shortening of the period of extraction is productive of a higher yield.^{5, 6} In the case of extraction for estrogens this does not appear to be a factor, the preliminary length of hydrolysis appearing to be the determining point in the yield.⁷ Nevertheless, whether estrogens or androgens are being extracted, the ability to complete an extraction during the working hours is important. Where inflammable or explosive materials are being used, the fire hazard is too great to allow an extraction to proceed throughout the night. We computed that seven liters of benzene can be passed through the urine in eight hours so that ten volumes of benzene, considering volume for volume, can be passed through the hydrolyzed urine. While the reduction of the time is not a factor in the quantitative yield of estrogen, it is definitely an important factor in producing a higher androgenic yield.

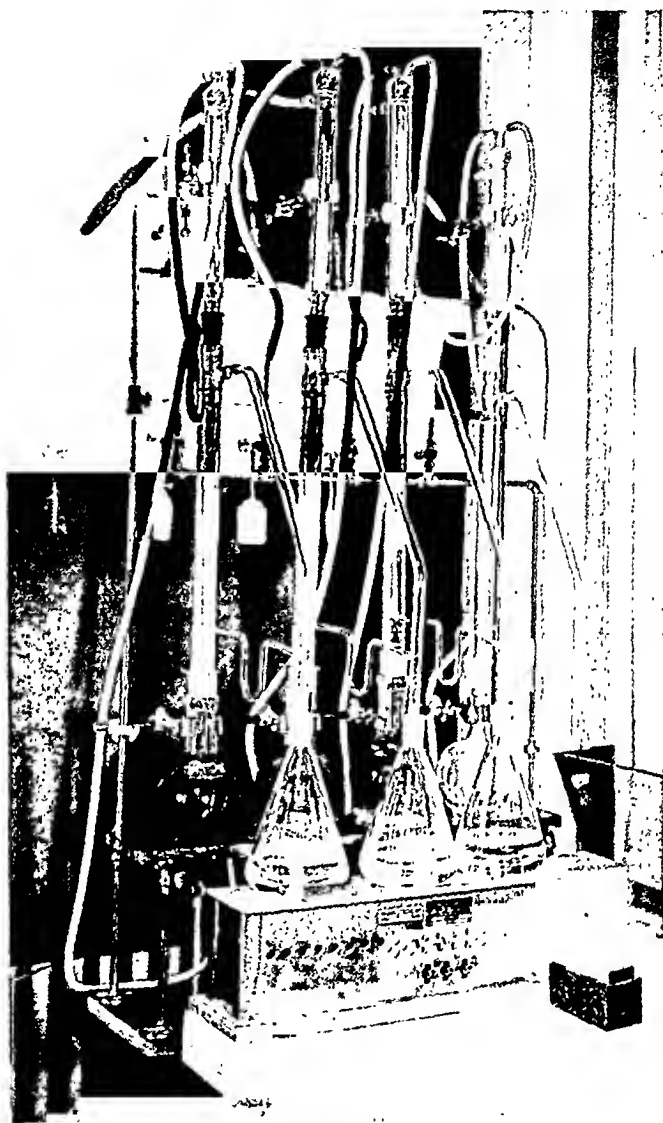


Fig. 3.—Photograph of extractors and extractor rack arranged as a battery of four extractors.

CONCLUSIONS

1. A simplified, economical, and efficient continuous extractor for estrogens and androgens is described.
2. An extractor rack to permit economy of space in arranging batteries of extractors is illustrated.
3. The principle of safety and efficiency in extraction is emphasized.

REFERENCES

1. Gallagher, T. F., Koch, F. C., and Dorfman, R. I.: Procedure for Quantitative Extraction of Sex Hormones From Urine, *Proc. Soc. Exper. Biol. & Med.* 33: 440, 1935.
2. Gallagher, T. F., Peterson, D. H., Dorfman, R. I., and Koch, F. C.: The Daily Urinary Excretion of Estrogenic and Androgenic Substances by Normal Men and Women, *J. Clin. Investigation* 16: 695, 1937.

3. Wilson, D., and Strickler, H.: Unpublished data.
4. Gallagher, T. F., Peterson, D. H., Dorfman, R. I., and Koch, F. C.: The Daily Urinary Excretion of Estrogenic and Androgenic Substances by Normal Men and Women, *J. Clin. Investigation* 16: 695, 1937.
5. Neudstadt, Rndolph: Photo-Colorimetric Method for the Determination of Androsterones in Urine, *Endocrinology* 23: 711, 1938.
6. McCullagh, D. Roy, and McLin, T. Roland: The Extraction of Androgens From Urine, *Endocrinology* 23: 711, 1938.
7. Grauer, R. C., and Saier, Eleanor: Unpublished data.

THE DETERMINATION OF CALCIUM IN URINE*

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THE estimation of calcium in the urine is usually more difficult than the estimation of serum calcium. This is due to the tendency of urates and other organic materials to coprecipitate with the calcium oxalate precipitate and thus interfere in the subsequent oxidimetric titration. By employing the direct acidimetric titration principle,^{1, 2} it was found that excellent results may be obtained in urine without resorting to ashing. In this procedure the calcium oxalate is converted to the carbonate or to the oxide by heat, which causes the decomposition of any adsorbed urates or other organic materials. To prove this point, comparison analyses were performed on urine, both directly and upon hydrochloric acid solution of the residue obtained by dry ashing. To prevent coprecipitation of magnesium and eliminate any possibility of phosphate precipitation, the pH of the solutions was adjusted to from 3.0 to 3.3 for precipitation of the calcium oxalate. The results are shown in Table I. Each value is the mean of two or more analyses on the same solution.

TABLE I

COMPARISON OF CALCIUM VALUES OBTAINED ON ASHED AND UNASHED URINE
(VALUES EXPRESSED IN MILLIGRAMS OF CALCIUM PER 100 ML. OF URINE)

UNASHED	ASHED
9.15	9.15
11.70	11.75
4.14	4.12
12.0	12.05
4.02	3.98
4.26	4.32

METHOD

For urine specimens without sediment. Two to 4 ml. of urine are pipetted into a 15 ml. pyrex ungraduated centrifuge tube. One milliliter of saturated ammonium oxalate is added. A drop of 0.04 per cent thymol blue indicator is added, and the pH is adjusted to from 3.0 to 3.3 by the use of 6 normal

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hydrochloric acid and ammonium hydroxide. The solution is allowed to stand for three hours. From this point on the method follows exactly that of Sobel and Sklersky for blood serum.¹ The oxalate is converted to carbonate by heating at 475° to 525° C. The carbonate is then dissolved in a hot 10 per cent boric acid solution and directly titrated with 0.01 N hydrochloric acid.

For urine specimens with sediment. In some urines, and in all urines after standing, a precipitate appears. This can be cleared up by boiling a representative sample of the urine plus sediment, or the entire specimen if the sediment is coarse-grained, preventing a representative sample from being taken, with concentrated nitric acid in the proportions of 10 ml. of acid to 50 ml. of urine. After boiling to one-half the original volume, the digest is diluted to known volume and a sample is analyzed as above.

Preparation of ashed urine specimens. To compare the calcium values obtained on ashed urine specimens to that of unashed urine, the following technique was employed: twenty-five milliliters of urine are pipetted into a platinum dish. The liquid is then evaporated off on a steam bath or in an oven at 100° C. overnight. The residue is then ashed in an oven at 500° to 600° C. until white. The ash is dissolved in 6 N hydrochloric acid and transferred quantitatively to a 25 ml. volumetric flask. Two milliliters of this solution are then used for analysis.

CONCLUSION

The direct acidimetric titration method for calcium is applicable to urine without ashing. This simplifies the methods for determining calcium in urine.

REFERENCES

1. Sobel, A. E., and Sklersky, S.: Direct Acidimetric Microtitration Method for Calcium, *J. Biol. Chem.* 122: 665, 1938.
2. Sobel, A. E., and Sobel, B. A.: Microestimation of Calcium in Serum, *J. Biol. Chem.* 129: 721, 1939.

THE SHORT PHENOLSULPHONEPHTHALEIN TEST OF RENAL FUNCTION*

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WITH THE TECHNICAL ASSISTANCE OF EDGAR FERGUSON, B.S.,
AND VICTOR GINSBURG, M.D.

IT MAY be safely stated that at the present time there is no altogether satisfactory test of kidney function. There is reason to believe that moderate or even advanced renal lesions may produce no modification in kidney function tests and that a kidney which is normal in every way may on occasion give a deceptively low reading. Evaluation of the patient's renal status means a consideration of his history, physical examination, examination of urine, blood, ocular fundi, and other routine examinations as well as the application of certain renal function tests. All the data must be considered and all the evidence weighed before any final judgment can be made and, even then, it must usually be made with caution unless the disease process is advanced. Don's conclusions, after using several different methods of testing renal function on 63 patients, is worth quoting.¹ "The lack of parallelism between the tests especially in the lesser grades of renal damage, was most disappointing. None of them appear to be capable of enabling the clinician to give a very much more accurate prognosis than is possible from the knowledge of the blood urea alone. However, if any of the tests show marked loss of renal function in the absence of a raised blood urea it would probably be best to give a guarded prognosis. A re-examination of the patient in three months might help; clinical examination will often furnish very valuable information and must still be regarded of the foremost importance. To conclude, the failure of the tests to agree closely with one another suggests either that they are not completely reliable or that different renal functions are being measured."

In view of the fact, therefore, that no one test of kidney function can provide a short cut to diagnosis, it seemed desirable to find one of the numerous tests now available which could be applied with a minimum of technical equipment and experience, with as little consumption of time as possible and would yet correlate fairly closely with more cumbersome methods. The criteria for a test of this sort seemed to be met by the fractional phenolsulphonephthalein test popularized by Chapman,² which can be completed in half an hour, requires a minimum of apparatus and reagents and can be used as a simple office procedure if necessary. Genitourinary surgeons have until very recently utilized the phenolsulphonephthalein test to a far greater extent than have internists, and it was they who first called attention to the importance of the

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early excretion of the dye.³ More recently, MacKay and Rytand have written: "The phenolsulphonephthalein test is superior to the blood urea concentration as a measure of the amount of functioning renal tissue."⁵

In an effort to repeat and confirm Chapman's experiments and to establish a series of satisfactory normals, the following project was carried out on the medical wards of the Kings County Hospital. Two hundred and three cases were studied in detail, of which 109 had obvious kidney damage. The fractional phenolsulphonephthalein test was correlated with the two-hour phenolsulphonephthalein, urea clearance, concentration tests, studies of nitrogen retention in the blood, and clinical data, although not all tests were done in every case. A detailed study of the sodium ferrocyanide test, as proposed by Stieglitz, was done simultaneously and has already been reported in detail in a separate paper.⁴

The technique of the test suggested by Chapman was followed by us with occasional modifications to be noted. The patient voids in the morning and drinks two glasses of water, noting the time. This may be done before reporting, but it is best to perform the entire test in the laboratory or office. One-half hour later 1.0 c.c. (6.0 mg.) of phenolsulphonephthalein solution is injected intravenously, and exactly fifteen and thirty minutes later the patient voids and the urine is collected. For the purposes of this experiment, one- and two-hour specimens were also collected, but this is unnecessary for routine examinations since no additional information is obtained by prolonging the test period. For use in the physician's office, when saving time is important, the total test period can be shortened from one hour to one-half hour in the following manner: When the patient arrives, give him two or three glasses of water to drink at the same time the injection is made and collect only the half-hour specimen if the patient cannot urinate at the end of fifteen minutes. The results are somewhat improved if the patient is told to drink a glass of water before leaving home. He should not void before the dye is given. We have found no significant differences between the two methods.

Vallery-Radot and his colleagues⁶ were favorably impressed by the short test and have abandoned longer procedures. In their tests all normal patients excreted 25 per cent or more within fifteen minutes. Readings of 15 per cent or less indicated some degree of failure of kidney function in almost all cases. They considered readings of about 20 per cent to be equivocal and repeated the tests. Part of the purpose of our experiment was to check these figures and to establish satisfactory normals for phenolsulphonephthalein excretion, and while we are not as yet wholly satisfied about this aspect of our work, we feel that our criteria are sufficiently accurate for most clinical purposes.

Normals.—One hundred and twenty-three short phenolsulphonephthalein tests were performed on 103 adult patients thought to be clinically free of renal disease or hypertension. In fourteen instances the phenolsulphonephthalein excretion after fifteen minutes was less than 25 per cent. In eight of these, one or more confirmatory tests (urea clearance, blood urea, ferrocyanide excretion) supported the conclusion that there was, temporarily at least, some impairment of renal function. It is possible, of course, that some of these patients will go on to clinical renal disease and that in any group of hospital patients presumably free of kidney involvement a small percentage would show some

changes in renal function tests. Of the remaining six patients, one showed a normal reading on repetition of the test. In five cases (4.9 per cent), in which there was no supporting evidence of renal impairment, the phenolsulphonephthalein readings were subnormal. The test gave a slightly higher percentage of normal figures in normal patients than any of the other tests, urea clearance, concentration tests, ferrocyanide clearance, blood urea, and two-hour phenolsulphonephthalein. In no case where the figure was low did the two-hour phenolsulphonephthalein test reveal normal figures. In two cases the figures were below normal for the quarter-hour excretion but were normal for the thirty-minute period.

Kidney Disease.—There were 21 cases of definite kidney impairment. The diagnoses in all cases were either chronic glomerulonephritis or nephrosclerosis. No tests were performed on patients with acute kidney disease. The fifteen-minute phenolsulphonephthalein test was 20 or below in nineteen of these. In one of the two others the figure was 22 per cent and in the other it was 25 per cent. In the former case 74 per cent of the dye was excreted in two hours. In the latter, the fifteen-minute figure was apparently normal but very little more dye was excreted in two hours, the total for that period being 35 per cent. The urea clearance was constantly diminished in all cases.

Hypertension.—There were 26 persons with early to moderate hypertension without clinical evidence of cardiac or renal failure. In eight of these the fifteen-minute phenolsulphonephthalein test showed diminished excretion. In only five was the urea clearance reduced below normal limits. This series is, of course, too small to be of much value, but it would seem to indicate that this test is a fairly sensitive method of detecting early renal changes in hypertension.

Congestive Heart Failure.—There were 20 patients with congestive heart failure. In all but two of these, during the stage of congestion there were low figures which improved as the cardiac function returned to normal. The urea clearance and the ferrocyanide excretion were correspondingly diminished. The blood nitrogen levels were not as consistently affected. Urine concentration tests were of little value during congestive heart failure.

There are few precautions needed in making or interpreting this kidney function test. We observed no reactions to the intravenous use of this dye, although Vallery-Radot, while temporarily using a somewhat different technique, did observe some. In the presence of liver damage the excretion is somewhat in excess of what one would expect, and borderline results should be interpreted conservatively and repeated. The explanation of this phenomenon probably lies in the fact that the liver usually excretes a portion of the phthaleins in the body through the biliary tract, and when there is failure of this mechanism, higher amounts appear in the urine. We have no experience with the test in uncomplicated anemia, but Chapman maintains that there is no significant effect of the latter on phenolsulphonephthalein excretion.⁷

Catheterization was rarely necessary. If the patient did not void after fifteen minutes, an extra glass of water was given and only the thirty-minute specimen was collected with little or no impairment of the test's value. Catheterization is, of course, an objection to any test which is to be used as an office procedure, but it may be necessary in prostatic enlargement or other conditions

in which there may be residual urine. The phenolsulphonphthalein test shares this disability naturally with any test which depends on the measurement of urinary constituents and is, therefore, necessarily limited in usefulness in cases of urinary retention. It should be noted that Macht⁸ maintains that phenolsulphonphthalein elimination may be delayed after the ingestion of magnesium sulfate for a period of as long as three to four hours. We have no experience with this.

Stieglitz⁹ cautions that "In rare instances some metabolic quirk causes chemical destruction of the dye within the body. If the urine contains no dye after injection, this is then no proof of renal failure." We have had no such experience but the caution should be borne in mind.

CONCLUSIONS

The short phenolsulphonphthalein test has been a very valuable aid to clinical diagnosis in our hands. The two-hour period has no advantage over the shorter periods. The positive value of the test is greater than its negative value. In other words, one is safe in saying that a patient who excretes 25 per cent or more phenolsulphonphthalein within fifteen minutes after its administration, or more than 35 per cent within thirty minutes, has normal kidney function. On the other hand, failure to secrete this amount has less diagnostic value. Borderline cases (17 to 24 per cent in fifteen minutes or 25 to 34 per cent in thirty minutes) should be evaluated with caution or the test should be repeated. Although definitely low reading almost surely means impaired kidney function at the time of the test, low readings should not be allowed to supersede clinical judgment.

SUMMARY

1. The difficulties of measuring renal damage by means of kidney function tests are described.
2. The short phenolsulphonphthalein (P.S.P.) test takes a half hour or less and is extremely useful. It is simple, requires a minimum of technical skill and equipment, and may be used as an office procedure. The two-hour phenolsulphonphthalein test has no advantages over the shorter test.

REFERENCES

1. Don, C. S. D.: Tests for Renal Function, *Brit. M. J.* 2: 54, 1937.
2. Chapman, E. M., and Halsted, J. A.: The Fractional Phenolsulphonphthalein Test in Bright's Disease, *Am. J. M. Sc.* 186: 223, 1933.
Chapman, E. M.: Further Experience With the Fractional Phthalein Test, *New England J. Med.* 214: 16, 1936.
3. Shaw, E. Clay: A Study of the Curve of Elimination of Phenolsulphonphthalein by the Normal and Diseased Kidneys, *J. Urol.* 13: 575, 1925.
4. Plotz, M., and Rothenberger, M.: Sodium Ferrocyanide Excretion in the Estimation of Renal Function, *J. LAB. & CLIN. MED.* 24: 844, 1939.
5. MacKay, E. M., and Rytand, D. A.: Significance of the Phenolsulphonphthalein Test of Renal Function, *Arch. Int. Med.* 55: 131, 1935.
6. Vallery-Radot, P., Delafontaine, P., Israël, R., and Porge, J.: L'épreuve de la phénolsulfonephtaléine en un quart d'heure, *Presse méd.* 44: 2009, 1936.
7. Chapman, E. M.: The Phthalein Test of Kidney Function in Anemia, *New England J. Med.* 213: 166, 1935.
8. Macht, D. I.: Concerning the Effect of Saline Purgatives on the Absorption and Excretion of Phenolsulphonphthalein, *J. Urol.* 7: 271, 1922.
9. Stieglitz, E. J.: Clinical Application of Renal Function Tests, *M. Clin. North America* 21: 281, 1937.

DEPARTMENT OF ABSTRACTS AND REVIEWS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

SULFAPYRIDINE, Effect of Sulfanilamide and, on Hemoglobin Metabolism and Hepatic Function, Watson, C. J., and Spink, W. W. Arch. Int. Med. 65: 825, 1940.

Sulfanilamide in customary therapeutic doses usually causes acceleration of the metabolism of hemoglobin characterized by an increase of urobilinogen in the feces and a varying increase in the reticulocyte percentage. The most marked acceleration of hemoglobin metabolism is represented by the unusual cases in which outspoken hemolytic anemia occurs. This condition is therefore to be regarded as a much more marked degree of a usual toxic effect of the drug. Limited data indicate that sulfapyridine has the same effect as sulfanilamide on hemoglobin metabolism.

The occurrence of macrocytic or normocytic, mildly hypochromic anemia after administration of sulfanilamide or sulfapyridine indicates a disturbance in hemoglobin formation in addition to increased hemolysis.

In many persons the administration of sulfanilamide in the usual doses is followed by some evidence of dysfunction of the liver, such as urobilinogenuria, elevation of the serum bilirubin, or outspoken jaundice. The last-mentioned condition is in part of the regurgitation type, as is evidenced by the frequent occurrence of a direct van den Bergh reaction. Sixteen cases of jaundice following sulfanilamide therapy have been encountered. But one instance has been noted in which jaundice followed use of sulfapyridine. Administration of sulfapyridine resulted in an elevation of the serum bilirubin in two cases in which frequent determinations were made before, during, and after administration of the drug. This is in contrast with the results in the group treated with sulfanilamide, in which significant elevation of the serum bilirubin was usual. Although the data on sulfapyridine are as yet too limited to permit definite conclusions, there is reason to believe that this drug may not be as disturbing to hepatic function as is sulfanilamide.

CARCINOMA, Cutaneous, Diagnosed Clinically Without an Autopsy. Result of Treatment in a Consecutive Series, Warren, S., Simmons, C. C., and Rea, S. L. J. A. M. A. 114: 1619, 1940.

Of 829 treated carcinomas of the skin not verified by biopsy, 84 per cent were followed for five years. There were 57 per cent three-year cures and 48 per cent five-year cures of all the tumors treated. If the cases lost and dead of intercurrent disease are counted as cures, the five-year cures would be 84 per cent; if they are entirely excluded, the percentage would be 76. Recurrences occurred in 13 per cent of cases showing primary healing followed one year or more.

Primary healing occurred in 94 per cent of the lesions followed one year or more. More than one-fourth of the deaths from cutaneous carcinoma occurred after primary healing; primary healing should not be considered a criterion of cure.

Failures are due largely to the use of very light filtered radon applied to the surface in inadequate dosage. X-rays or radium used at a distance, as is the more general practice today, would have given greater depth doses and probably have given better results.

VULVA, Benign and Malignant Tumors of, Folsome, C. E. J. A. M. A. 114: 1499, 1940.

Metaplasia and malignant change in apparently innocuous vulvar tumors are inadequately emphasized. Papillomas, sebaceous cysts, pigmented moles, leiomyofibromas, vulvar breast tissue, and sweat gland adenomas are some of the benign vulvar tumors which may, at a later date in a woman's life, degenerate to vulvar malignancy.

Treatment and care of the patient presenting herself with a vulvar cancer are tending to become more rational in the light of present anatomicopathologic knowledge of vulvar cancers. Vulvar surgery combined with resection of superficial and deep inguinal lymph nodes, the so-called Basset technique, with modifications as outlined by Taussig, continues to be the best treatment for carcinoma of the vulva.

By stressing periodic pelvic examinations, cancer educational programs may materially assist in preventing cancer of the vulva. By paying attention to simple lesions and the benign tumors of the vulva, the physician may prevent carcinoma of the vulva.

COLLOIDAL GOLD, A Method of Preparation, Bartholomew, R. J., and Gent, N. L.
Australian J. Exper. Biol. & M. Sc. 18: 89, 1940.

A method is described for the preparation of colloidal gold sols for use in the Lange test by the reduction of gold chloride with sodium citrate.

A sol of correct sensitivity can be prepared (a) by using a suitable amount of citrate in the preparation, (b) by adding acid to an initially "hard" sol, (c) by adding alkali to an excessively sensitive sol, (d) by mixing suitable proportions of a "hard" sol and an excessively sensitive sol. Method (b) is preferred for routine use, and a technique for preparation by this method is described.

Not a single failure has been encountered in preparing citrate-reduced sols, and the sols have been found so uniform in behavior that it seems unnecessary to use a "paretic" fluid for standardization.

The method follows:

Into a liter flask put 675 ml. distilled water, close the flask with a ground-in reflux condenser, and bring the water to the boil over a fairly large flame. Remove the condenser and add 7 ml. of 1 per cent gold chloride solution followed by 17.5 ml. of 1 per cent sodium citrate solution. Replace the condenser and continue the boiling for fifteen minutes. Remove from the flame and allow to cool.

To one 50 ml. portion of the sol add nothing, and to three other 50 ml. portions add 0.05 ml., 0.10 ml., and 0.15 ml., respectively, of N/10 hydrochloric acid. Set up each mixture by the conventional technique against a known "paretic" cerebrospinal fluid and against a known "normal" fluid, then allow the mixtures to stand overnight. Select the mixture which gives the strongest reaction with the paretic fluid, yet does not give a reaction greater than 0 0 1 1 0 0 0 0 0 with the normal fluid, then add the corresponding quantity of acid to the main bulk of the sol, thoroughly agitating the mixture during the addition.

TYPHOID, Isolation of Paratyphoid, Dysentery and, From Feces and Urine, Ruys, A. G.
Brit. M. J., April 13, p. 606, 1940.

This comparative study indicates that in cases of typhoid fever it is advisable to use the media of Wilson and Blair and of L. Muller; in paratyphoid B, those of Muller and Ruys; and in the enteric form of the disease, those of Kauffman and Ruys. Endo's agar should not be neglected because it may give a positive result one day earlier than any of the other media.

In bacillary dysentery both Leifson's desoxycholate-citrate agar and Endo's agar should be used.

SULFAPYRIDINE, Evaluation of the Clinical Toxicity of Sulfanilamide and, Brown, W. H., Thornton, W. B., and Wilson, J. S. J. A. M. A. 114: 1606, 1940.

The vomiting caused by the administration of sulfapyridine appears to be of both local and central origin. Mucilage of tragacanth and nicotinic acid are of value in combating the vomiting and other mild toxic reactions.

Although methemoglobinemia contributes to cyanosis in a small number of cases, sulfhemoglobinemia has not been encountered.

The serious toxic manifestations have been found twice as frequently in the series treated by sulfapyridine as compared with the series on sulfanilamide.

Oliguria, hematuria, pain in the costovertebral angle, and anuria are serious and fairly common complications of intensive sulfapyridine therapy, particularly when the concentrations in the blood are high. These sequelae are related to the insolubility of acetylsulfapyridine, which precipitates in characteristic crystals in acid and alkaline urines. For this reason, the fluid intake should approximate 3,000 c.c. or more in twenty-four hours, and the urine volume in a like period should not be allowed to fall under 1,000 c.c.

Although leucopenia occurs more frequently in the sulfapyridine series and especially when the dosage is heavy, it is also encountered in both series and may occur when the doses are small or treatment is of short duration.

Acute hemolytic anemia with icterus was encountered only in the cases in which sulfanilamide was administered. Both series comprised instances of gradual and suddenly developing anemia.

Three cases of granulocytopenia were encountered in 100 cases in which sulfapyridine was given. All three occurred in cases in which treatment was maintained for longer than fifteen days at low blood concentrations.

Patients treated with intensive doses and in whom high blood concentrations of sulfapyridine were attained showed a very high incidence of serious toxic reactions.

Drug rashes and fevers are useful signs of toxicity, but serious toxic complications may occur without these warnings.

Sulfapyridine may remain in the liver in small quantities for as long as forty days after chemotherapy has been terminated. No conclusion has been reached as to whether sulfapyridine causes hepatitis.

Sulfapyridine is essentially more toxic than sulfanilamide.

GLUCOSE TOLERANCE, Reverse or "Paradoxical" Blood Sugar Response to Ingested Glucose, Wheelon, H. *Endocrinology* 26: 743, 1940.

Laboratory and clinical findings are presented on 40 patients, each of whom showed a reverse or "paradoxical" blood sugar response to the ingestion of glucose, and a sugar-free urine.

The average fasting blood sugar level for this series was 140 mg., or a figure indicative of mild hyperglycemia. Blood sugar levels of 70 mg. or less were found twenty times on 10 patients during the glucose tolerance test. The initial blood sugar response of 14 glucose tolerance tests performed on 7 patients subsequent to the initial tests was an average elevation of 33 mg. above the fasting level, followed by a maximum depression of 19 mg. below the fasting reading. Five patients in the series of 7 initial tests developed hypoglycemic sugar levels ten times. A like number of patients developed hypoglycemic levels eight times in the fourteen subsequent series of tests. Two patients only, however, developed hypoglycemic levels in each of the two series.

Ninety-nine physical, and 248 chemical, a total of 347 determinations were made on the 40 patients in addition to those incident to the glucose tolerance test, urine and Wassermann examinations. A total of 39, or 40 per cent, of the 99 physical determinations lay beyond the limits of normal variation. A total of 107, or 43 per cent, of 248 chemical determinations lay outside the range of figures assumed to represent "normal variations."

Seventy-two clinical diagnoses, aside from those established by laboratory procedures, were made on the 40 patients. Diagnoses referable to disturbances of the nervous system were made on 29, or 73 per cent, of the 40 patients.

A correlation of the various findings shows that each of the 40 individuals studied had an average of 5.5 deviations from normal standards, in addition to the primary one, or reverse response to the ingestion of glucose.

It may be concluded that a rather large number of physical and chemical abnormal variations and diagnosable clinical states are found associated in various degrees with

reverse or "paradoxical" blood sugar responses to the ingestion of glucose. It may be concluded also that the medical management of patients showing reverse glucose responses, as demonstrated on 7 patients, results in the amelioration of the symptomatology and a return of the glucose response toward the usual normal.

SODIUM SULFAPYRIDINE, Use of, by Hypodermoclysis, Taplin, G. V., Jacox, R. F., and Howland, J. W. J. A. M. A. 114: 1733, 1940.

Sodium sulfapyridine has been used by hypodermoclysis in more than 50 cases of pneumonia and numerous other conditions for which sulfapyridine was indicated but in which oral administration was difficult or impossible. The drug was given in from 0.3 to 0.7 per cent solution in physiologic solution of sodium chloride and no local reactions were observed in any of the cases. More than 1,100 Gm. of the drug have been given by this method.

This route of administration is advocated when the drug is not tolerated by mouth or is poorly absorbed from the gastrointestinal tract and when a high sustained concentration in the blood is imperative.

SULFANILAMIDE, Absorption of, as an Index of Blood Flow in the Intestine of Man, Stead, E. A., and Kunkel, P. Am. J. M. Sc. 199: 681, 1940.

The blood flow in the human intestine has been investigated by determining the appearance time and the concentration curve in the blood of free sulfanilamide when a known amount of sulfanilamide in solution is introduced directly into the small intestine or colon.

Under basal conditions a blood level of 6 to 8 mg. per 100 c.c. of free sulfanilamide was obtained in ten to thirteen minutes when 300 c.c. of a 1 per cent solution of sulfanilamide were placed in the duodenum.

Heating the body or inducing collapse in an individual with postural hypotension did not alter the curve of sulfanilamide in the blood. This is interpreted as indicating that the basal blood flow to the small intestine was low and that the rapid appearance of sulfanilamide in the blood was the result of its rapid diffusion.

Under basal conditions sulfanilamide was absorbed much more slowly from the large bowel. When 300 c.c. of a 1 per cent solution of sulfanilamide were given rectally, the blood level rose to between 2.4 and 4 mg. per 100 c.c. at the end of one and one-half hours.

Pitressin failed to influence the rate of absorption of sulfanilamide when the substance was given by duodenal tube. Pitressin caused a delay in absorption when the sulfanilamide was placed in the large bowel.

The rapid absorption of the sulfanilamide in the collapse induced by the upright position in a person with postural hypotension suggested that even in shock sulfanilamide diffuses so rapidly that it is adequately absorbed once it is present in solution in the small intestine. Dilatation of the stomach and closure of the pylorus may, however, prevent the drug from reaching the small intestine.

SULFATHIAZOLE, Crystalline Concretions in the Renal Tubules Following, Pepper, D. S., and Horack, H. M. Am. J. M. Sc. 199: 674, 1940.

Concretions obstructing the renal tubules and in the pelvis and bladder were discovered at autopsy in a patient following treatment with sulfathiazole. On analysis the material was composed of a derivative of sulfathiazole. The gross and histologic findings in the kidneys were identical with those described by others as occurring in experimental animals. The probable etiologic factors influencing the site of the precipitation of sulfapyridine and sulfathiazole crystals are discussed. Because of the intrarenal precipitation of sulfathiazole, it is felt renal complications resulting from the use of this drug may be more serious than those following the use of sulfapyridine.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor,
Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

Electrocardiography*

THIS book is intended to serve as an introduction to the study of electrocardiography and is designed for the use of the general practitioner, the medical student, and others who are not primarily cardiologists. The use throughout the book of the standard nomenclature advocated by the American Heart Association is to be commended.

The book is an atlas of one hundred electrocardiographs with clinical diagnosis and interpretations. There is an interlarding of textual material. Although the authors state that the tracings have been carefully standardized, the reviewer would have preferred to have the standardizations given with each tracing, instead of being confined to the first. It is felt, however, that the aim of the authors is achieved very satisfactorily, and that the book may be recommended to the audience for which it was intended.

Vitamin E†

IN APRIL, 1939, a symposium was held on vitamin E in London. Taking part in this international, but predominantly British, discussion were many leaders in the field. The proceedings were edited by A. L. Bacharach and J. C. Drummond. The monograph constitutes a valuable summary of our knowledge in a highly important biochemical field.

The little book falls naturally into three subdivisions: the chemical structure and properties of tocopherol (vitamin E); the physiologic action of vitamin E and the consequences of vitamin E deficiency; and the clinical and veterinary uses of wheat germ oil and vitamin E preparations. In the eighteen years since the discovery of an antisterility vitamin its structural formula has been established and it has been synthesized. In addition to the biological estimation, there are four chemical methods for its analysis. The therapy of avitaminosis E is fast becoming rationalized. Although much work remains to be done before the last word has been said on vitamin E, this book brings together in a concise form the great deal that has already been accomplished.

*Electrocardiography. By Chauncey C. Maher, B.S., M.D., Assistant Professor of Medicine, Northwestern University and the Montgomery Ward Medical Clinics; Attending Internist and Chairman of Department of Medicine at the Cook County Hospital, Chicago, Ill.; and Paul H. Wosika, M.D., M.S., Instructor in Medicine, Northwestern University and the Montgomery Ward Medical Clinics; Attending Physician at Passavant Memorial Hospital, Associate in Medicine at the Cook County Hospital, Chicago, Ill. Cloth, ed. 3, 334 pages, \$4.00. A William Wood Book. Williams & Wilkins Co., Baltimore, Md., 1940.

†Vitamin E. A symposium held under the auspices of the Food Group (Nutrition Panel) of the Society of Chemical Industry on April 22, 1939, at the School of Hygiene and Tropical Medicine, Keppel Street, London, W.C.1, England. Cloth, 88 pages, \$2.00. Chemical Publishing Co., Inc., New York, 1940.

CORRESPONDENCE

Wichita, Kansas,
October 9, 1940.

The Editor,
JOURNAL OF LABORATORY AND CLINICAL MEDICINE.
Dear Sir:

Perhaps some of the readers are tired of being unable to think while shaking a Kahn rack full of test tubes in their Kahn shaking machine. Perhaps, at the same time, they do not wish to throw away their Kahn racks and purchase the new rubber coated ones, which are silent in operation. Here is a way to silence the old racks.

A bottle of "Rug Sta," which is manufactured by the makers of Old English Wax, will do the trick. Merely take an old tooth brush and lay the "Rug Sta" on in a thick smooth coat. It is white when wet and colorless when dry. Two coats will usually suffice to make a silent test tube rack out of the noisiest of them.

JACK G. STEELE,
Midwest Laboratory.

Washington, D. C.
September 16, 1940.

The Editor,
JOURNAL OF LABORATORY AND CLINICAL MEDICINE.
Dear Sir:

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PROGRESS

PHYTOTOXIC REACTIONS OF SOME BLOOD SERA*

WITH BIOMETRIC ANALYSES

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BALTIMORE, MD.

INTRODUCTION

NEARLY twenty years ago, while studying the general laws underlying physiologic and pharmacologic reactions, one of us (D. I. M.) conceived the idea of making comparative studies of the effect of various drugs and chemicals on living plant protoplasm in contrast to living animal test objects. By pharmacology, as it is taught in most of our medical and research institutions, is meant the investigation of the effects of drugs and poisons on living animals. Strictly speaking, it is *zoopharmacology*. However, just as the domain of physiology is subdivided into *zoophysiology*, i.e., study of physiologic functions of animals, and *phytophysiology*, or plant physiology, or study of physiologic functions of plants, so can the science of pharmacology be subdivided into *zoopharmacology* and *plant pharmacology*, or study of the effects of drugs and poisons on living plants. Initial studies of this type, carried out by one of us (D. I. M.), led to such interesting findings that as a result there was developed a new department of science, to which was given the name of "*phytopharmacology*." A number of the discoveries made in the evolution of this branch of pharmacology have a direct bearing on clinical medicine and in the present paper we shall describe and explain some of these developments.

The effects of drugs and poisons on plant tissues may be studied in various ways. Thus the plant physiologist entering this field may study the effects of chemicals on germination of seeds, on growth of roots and stems, on flowering

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and flowers; the effects of various chemicals on geotropic and heliotropic properties of plants, on processes of respiration and transpiration, on growth of yeasts and fungi, on behavior of chloroblasts, on protoplasmic streaming, photosynthesis, oxidation and reduction phenomena, etc. For practical pharmacologic purposes it was found that quantitative phytopharmacologic studies could be most conveniently made by measuring the growth of roots and sometimes of stems of certain seedlings reared in artificial plant physiologic nutrient media. Growth of such seedlings in different plant physiologic solutions containing a definite proportion of the salts necessary for normal growth and of other seedlings of the same type reared in similar solutions to which small amounts of unknown drugs had been added, could then be quantitatively compared. For such studies various plant physiologists have found the seeds of the *Lupinus albus* very useful, and we and our collaborators have employed them extensively in our phytopharmacologic researches.

STUDIES ON COCAINE

The earliest phytopharmacologic studies, carried out by Macht and Livingston,^{2, 3} dealt with the pharmacodynamic effects of the alkaloid cocaine and its products of hydrolysis on growth of *Lupinus albus* seedlings under standard ecologic conditions. It is well known that the cocaine molecule can be easily split by hydrolysis into three components, one a complicated nitrogenous base known as ecgonin, another, a molecule of a simple aromatic benzoic acid, and the third, a molecule of methyl alcohol; and that cocaine itself, or in the form of its simple salts, is a powerful poison for animals and animal tissues. Ecgonin is not as toxic for animals as cocaine itself; small doses of methyl alcohol are certainly not toxic for most lower animals; and benzoic acid as such, or in the form of its sodium salt, is practically nonpoisonous. A study of cocaine and its products on living seedlings of *Lupinus albus* by methods described elsewhere and to be discussed at length in the present paper, revealed that cocaine itself is not extremely poisonous for plant test objects. On the other hand, sodium benzoate, which is practically nonpoisonous for animal tissues, was very toxic for living plant protoplasm. A 2 per cent concentration of cocaine hydrochloride in the plant physiologic solutions was required to inhibit growth of *Lupinus albus* seedlings completely, while a 0.007 per cent solution of sodium benzoate accomplished the same result. It thus became apparent that while cocaine was much more poisonous for living animal tissue than for plant protoplasm, one of its components, sodium benzoate, was far more toxic for plant tissue than for living animals. This curious observation suggested that the presence of some toxic substances, in quantities not pronounced enough to affect animals, might be detected by means of living plants. Accordingly, one of us (D. I. M.) debated the feasibility of employing plants to determine the toxicity of certain bloods which could not be demonstrated by any other biological or physicochemical method. Such inquiry has been attended by more success than was ever anticipated.

STUDIES ON MENOTOXIN

The first phytopharmacologic studies undertaken on blood toxins were suggested by certain historical considerations. The belief is popular among all

races, ancient, medieval, and modern, that a woman at time of her menstruation is "unclean," using the word in a transitive sense as contaminating. Such a person was supposed to defile everything with which she came in contact. Thus, for instance, flowers touched by a menstruating woman withered; fruit that she canned became spoiled; dough that she kneaded failed to rise; and wine that she expressed from grapes soured. No careful investigation was made of the subject until 1920, when Professor Shiek observed that beautiful, freshly cut flowers handled by a menstruating woman assistant commenced to fade soon after she had touched them. Reporting this circumstance and the similar results he had obtained in experiments with flowers in the hands of other menstruating women, Dr. Shiek expressed the hope that some one would undertake to study the subject in a thoroughly scientific quantitative manner. Such a scientific investigation was carried on by Macht and Lubin,⁴ who demonstrated quantitatively that small amounts of menstrual blood sera, as compared with normal sera of the same individuals, inhibited the growth of *Lupinus albus* seedlings. Moreover, the presence of this toxic substance, named "menotoxin" (by D. I. M.), was demonstrated by similar phytopharmacologic methods in the saliva, milk, urine, tears, and particularly the sweat, of menstruating women. These studies, published seventeen years ago, were later supplemented by those of Macht and Davis,⁵ who showed that chemically menotoxin is probably akin to oxycholesterin. Their findings, which other investigators confirmed by different means, are now of special interest in connection with phytopharmacologic studies on the nature of the sex hormones, also found to be closely related in chemical structure to cholesterol. Most important of all, however, the first studies on menotoxin, instigated by the interest in medical history (D. I. M.), led to discovery of toxic reactions in the blood of patients suffering from various pathologic conditions. In the present paper we purpose to recapitulate the results already obtained in studies made on various types of blood sera and to describe the research we are now conducting and the phytotoxic reactions we discovered in bloods from other diseases.

TOXIC SERA

The phytopharmacologic method will be described in detail in the second part of this paper. Briefly, it consists of studying the root growth of *Lupinus albus* seedlings in normal plant physiologic solutions and comparing it with that of similar seedlings in the same kind of solution, to which had been added definite concentrations of the blood sera or plasma to be examined. The average root growth of *Lupinus albus* seedlings in twenty-four hours under standard conditions of temperature and light in a solution containing an unknown serum or chemical divided by the average increment of control roots is known as the phytotoxic index or index of growth, which may be expressed by the formula,

$$\text{Index} = \frac{X}{N}.$$

The average phytotoxic index of root growth of *Lupinus albus* seedlings in plant physiologic solutions containing small amounts of blood sera from hundreds of

normal individuals was found to be 70 to 75 per cent. Blood sera obtained from a number of women at onset of catamenia yielded an average phytotoxie index of 51 per cent. One of the most remarkable findings made in early phytopharmacologic studies was that obtained during investigation of sera from persons with various anemias. Blood sera of patients with pernicious anemia, male and female, definitely inhibited growth of *Lupinus albus* seedlings.^{6, 7} Specimens from the first 50 patients examined yielded an average index of 44 per cent, a reading considerably lower than that given by menstrual blood. Such a phytotoxie effect was exerted not only by the blood but also by the spinal fluid⁸ and stomach washings⁹ from persons with pernicious anemia. Sera of certain other diseases, listed in Table I, also exhibited a characteristic phytotoxicity.

TABLE I
TOXIC SERA

KIND OF BLOOD SERUM	AVERAGE NUMBER OF CASES	PHYTOTOXIC INDEX (%)
Normal human	500	75
Menstrual	50	51
Pernicious anemia	50	44
Aplastic anemia	10	56
Pemphigus	200	59
Leprosy	22	47
Trachoma	100	48
Hodgkin's disease	10	60

TABLE II
OTHER ANEMIAS

KIND OF BLOOD SERUM	PHYTOTOXIC INDEX (%)
Severe secondary anemia	65 to 69
Carcinoma	70
Pellagra	70
Hemolytic jaundice	74
Obstructive jaundice	71
Malaria	70
Mononucleosis	80
Banti's disease	75
Lymphatic leucemia	68
Myelogenous leucemia	78
Sprue	60

Sera from patients with aplastic anemia gave an index of 50 per cent; those obtained from persons with leprosy, a reading of 47 per cent; from trachoma, 48 per cent; from Hodgkin's disease, 60 per cent. Most important of all, blood sera from the baffling skin disease, pemphigus, yielded an average phytotoxie index of 59 per cent. These phytotoxie reactions assume a profound significance when considered with the findings obtained in studies of sera from other forms of anemia and dermatoses.^{10, 11} Table II exhibits the findings obtained with sera from secondary anemias, from the anemias of malignant disease, from various types of leucemia and certain other pathologic conditions. Thus was

established the availability of a test for differentiating between pernicious anemia and other types of anemia, between pemphigus and other forms of skin disease simulating it, and between trachoma, regarded by many as a systemic disease, and other varieties of conjunctivitis and localized eye disease.¹²

TABLE III
NONTOTOXIC SERA

KIND OF BLOOD SERUM	PHYTOTOXIC INDEX (%)
Syphilis	81
Tuberculosis	78
Scarlet fever	79
Measles	80
German measles	80
Varicella	80
Eclampsia	75
Postpuerperal (12 weeks)	80

Of equal interest were the *negative* findings derived from phytopharmacologic study of sera from certain grave pathologic conditions, i.e., syphilis, tuberculosis, scarlet fever, and other exanthemas, and also eclampsia. The phytotoxic indices of some of these nontoxic sera are shown in Table III. Tables I to III are obviously of clinical interest and throw some light on the etiology of certain diseases, such as pemphigus, pernicious anemia, trachoma and eclampsia. The phytopharmacologic test is also helpful in differentiating some pathologic conditions having certain points of resemblance. Thus, for instance, one of us by this method has differentiated between syphilis, tuberculosis, and leprosy. Further data on the subject may be found elsewhere. In this connection it is interesting to note that the average phytotoxic index of normal human blood specimens, 70 to 75 per cent, is practically the same as that obtained with sera of other mammalia, small and large, and of most fish and other cold-blooded animals with the exception of reptiles.

TABLE IV
SERA FROM VIRUS DISEASES

KIND OF BLOOD SERUM	ANIMAL INOCULATED	PHYTOTOXIC INDEX OF CONTROL (%)	PHYTOTOXIC INDEX OF VIRUS SERUM (%)
Fowl pox	Chicken	71	80
Vaccinia	Rabbit	73	80
Vaccinia	Rat	67	78
Herpes simplex	Rabbit	72	84
Virus fixé	Rabbit	75	61
Virus fixé	Guinea pig	81	65
Virus III	Rabbit	72	84
X-virus	Cat	70	65
Rous' tumor	Chicken	66	92
Shope's fibroma	Rabbit	77	96
Infectious myxomatosis	Rabbit	75	85
Rivers' lymphocytic choriomeningitis	Guinea pig	79	102
Poliovirus	Monkey	61	62
Tetanus toxin alone, 86 per cent	Rabbit	75	63
Strychnine nitrate, 93 per cent	Rabbit	77	61

ATOXIC SERA

The negative results obtained with sera of certain diseases listed in Table III suggested an investigation which was conducted by Macht and Gardner,¹² who studied blood specimens from various animals experimentally inoculated with a number of virus diseases.

Blood was taken from such animals before experiments were begun and later after the virus diseases were fully developed. It was found that sera from animals inoculated with the virus diseases yielded phytotoxic indices much higher than normal blood specimens from the same animals. Bloods from animals inoculated with virus fixé or virus hydrophobia were the only exception, and the increased toxicity of such specimens was shown to be due to convulsions produced by the virus because similar findings were made after injection of small amounts of strychnine and tetanus toxin (see Table IV).

BLOOD SERA FROM PEMPHIGUS, X-RAYED, AND PSYCHOTIC PATIENTS

During the past five years the phytopharmacologic test has been a routine procedure in various researches made in these laboratories. Such investigation has been made on the comparative toxicity of various synthetic inorganic and organic compounds, the growth hormones, the influence of vitamins on plant growth, the pharmacologic effects of sex hormones on growth of plants, and a search for toxins in various diseases of the blood. Special studies, described here in detail, were made on (1) blood sera from animals and human beings after their exposure to x-rays, (2) the toxicity of pemphigus blood sera, and (3) blood sera from patients with various organic and functional psychoses.

The first problem undertaken concerned the effects of prolonged administration of x-rays and had a direct bearing on the so-called roentgen sickness. Repeated experimentation revealed that from twenty-four to forty-eight hours after exposure of various animals to therapeutic doses of x-rays, a toxic substance was developed in their blood which could be demonstrated phytopharmacologically.¹⁴ As compared with normal specimens that had been drawn from the same animals before treatment with x-rays, blood sera obtained afterward were quite toxic, but the toxicity varied with the region of the body irradiated. When these tests on normal animals had been completed, a similar series was begun with human beings, from whom blood sera were obtained both before and after their treatment with x-rays for malignant and nonmalignant pathologic conditions. It was found that specimens from patients who had been subjected to such exposure definitely inhibited growth of *Lupinus albus* seedlings. Further investigation revealed the fact that not only sera from patients treated with x-rays but also those from apparently healthy physicians, technicians, and attendants in roentgenologic clinics were definitely toxic for *Lupinus albus*, as compared with control specimens from unexposed normal individuals. A number of these findings have been published, but the subject itself, which is of great clinical importance, is still under investigation. Table V shows the striking data already obtained.

TABLE V
EFFECT OF X-RAYS ON BLOOD OF CLINICAL PATIENTS

CASE NO.	DIAGNOSIS	PHYTOTOXIC INDEX		TIME ELAPSING BETWEEN X-RAY TREATMENT AND TAKING OF SPECIMEN	DOSAGE EMPLOYED (r.)
		BEFORE TREAT- MENT (%)	AFTER TREAT- MENT (%)		
N 17	Carcinoma of breast	73	45		200
N 17	Carcinoma of ovary	74			No x-ray
M.B.M.	Hypertrophied tonsils	72	37	24 hours	333
		72	72	8 days	
105391	Carcinoma of breast	68	42	48 hours	265
180902	Carcinoma of cervix	52	45	48 hours	156
181248	Carcinoma of breast		47	48 hours	160
174802	Carcinoma of prostate	79	46	24 hours	260
175925	Syringomyelia	73	56	24 hours	101
159409	Myoma	61	41	24 hours	270
174976	Carcinoma of submaxillary gland	66	58	24 hours	198
169275	Eustachian salpingitis	69	30	24 hours	129
176084	Hypertrophic nasopharyngitis	72	41	24 hours	160
183574	Carcinoma of breast	84	40	24 hours	202
170813	Carcinoma of breast	66	58	48 hours	194
181423	Chancre of tongue	69	64	24 hours	2 x 160
Mrs. Y.	Carcinoma of breast	68	32	24 hours	300

We still conduct another phytopharmacologic study of pathologic blood sera, initiated a year ago by Dr. Max Hayman, then a resident physician at Springfield State Hospital, Sykesville, Md., and one of us (D. I. M.). This research consists of a systematic examination of blood sera from various types of functional and organic psychoses. A complete presentation of the findings will be published in due time after the blood sera from a larger number of cases have been studied. Such an investigation is in progress. Table VI shows the results obtained after repeated examination of sera from eight psychotic patients. The case of one such patient will be described in detail here.

TABLE VI
PHYTOTOXIC INDICES OF SOME PSYCHOTIC BLOOD SERA

PATIENT	DIAGNOSIS	PHYTOTOXIC INDEX (%)
E.S.	Schizophrenia, catatonic hypokinetic type	44
H.R.	Schizophrenia, catatonic hypokinetic type	42
F.N.	Schizophrenia, catatonic hyperkinetic type	58
D.H.	Schizophrenia, catatonic hyperkinetic type	58
C.H.	Involuntional melancholia type	52
M.R.	Involuntional melancholia type	50
B.B.	Manic-depressive type	54
J.H.	Manic-depressive type	57

By far the most extensive phytopharmacologic study, which is still in progress, has been that made by the dermatologist, Dr. Isaac R. Pels, and one of us (D. I. M.) on pemphigus blood sera, which exhibited more toxicity than specimens obtained from all other skin affections, with the exception of leprosy. In fact, the phytopharmacologic test has become not only a valuable aid in diagnosing and differentiating this strange malady from dermatitis

herpetiformis and other dermatoses, but also an important contribution to the etiology of pemphigus. It has been most useful in establishing a diagnosis in the early stages of pemphigus. Although pemphigus is a rare disease, Machl and Pels up to the present time have examined phytopharmacologically more than 2,600 specimens taken from various skin diseases and forwarded to them for differential diagnosis by physicians in the United States and Canada. Routine phytopharmacologic examination of these blood sera has resulted in a positive diagnosis of pemphigus in fully 90 per cent of the cases, a diagnosis which was confirmed by clinical findings as the disease progressed. This figure in itself is incontrovertible proof of the value and reliability of the phytopharmacologic test for this disease. Because of the growing importance and practical value of the phytotoxic reaction in routine examination of blood sera from various diseases, and because of the difficulties some clinicians experience in carrying out the test, we purpose to describe it in considerable detail and to evaluate its reliability in the light of the latest biological and biometric information available.

METHOD OF EXPERIMENTATION

The method of detecting toxic reactions in blood sera of pernicious anemia, pemphigus, and other pathologic conditions consists of determining quantitatively the increment in length of the single, well-defined, straight roots of seedlings of *Lupinus albus*, after their growth for twenty-four hours in a plant physiologic (Shive) solution containing a small amount of the blood serum to be tested. The average root length of ten or more such seedlings is measured, and the plants are immersed in a suitable plant physiologic nutrient solution containing all the salts or electrolytes necessary for perfect growth. Seedlings are grown in such solutions for twenty-four hours under standard conditions of light and temperature, after which the average increment in root length is determined. These seedlings grown in Shive solution are compared with similar seedlings placed for the same number of hours in the same kind of plant physiologic solution containing, in addition, a definite quantity of an unknown chemical, blood serum, or other substance to be tested. When a pathologic specimen of blood is examined, the growth of seedlings is determined not only for one group in plant physiologic solution alone, but also for another in Shive solution containing a small percentage of normal blood serum. It is customary in all phytopharmacologic work to employ 1 per cent solutions of blood sera.

To make a correct phytopharmacologic test a great deal of training is required not only in ordinary pharmacologic methods, but also in laboratory technique pertaining to plant physiology. Beginners who anticipate success with the first attempt at such experimentation will probably be disappointed. Without previous practice even experienced laboratory workers have occasionally been unable to germinate the seeds, much less to raise the seedlings and measure them in phytopharmacologic tests. In this highly specialized work, as in any other complicated laboratory technique, negative results in the hands of a tyro count for little or nothing.

In examining specimens for pemphigus toxin, fresh blood serum is almost invariably employed in making the test although, as Macht and Grumbein have pointed out,¹⁵ dried specimens of whole pemphigus blood or of blood serum retain much of the toxin and may be used for phytopharmacologic examination when necessary. Occasionally, serum from bullae is also tested for the pemphigus toxin. A 1 per cent solution is prepared by dissolving the serum in equal parts of distilled water and Shive plant physiologic solution,¹⁶ which consists of 10.4 c.c. of a 0.5 molar solution of chemically pure calcium nitrate, 30 c.c. of a 0.5 molar solution of chemically pure magnesium sulfate, and 36 c.c. of a 0.5 molar solution of diacid potassium phosphate (KH_2PO_4), to which is added enough distilled water to make one liter. In testing body fluids for clinical purposes, it is absolutely necessary to use salts of the highest chemical purity. Even the test tubes employed in such work should be of a special type, i.e., they should be made of hard or nonalkaline glass, in order that all conditions of experimentation may be rigidly controlled and standardized. The hydrogen-ion concentration of a mixture of equal parts of distilled water and Shive solution is usually about 5.4. When blood serum is added to make a 1 per cent solution, the pH shifts toward the alkaline side and varies from 5.4 to 5.6. Previous publications have shown that such slight variations in pH have little effect on the growth of seedlings immersed in the solutions and are certainly not sufficient to account for differences in toxicity between specimens of serum from both normal and diseased persons. As a matter of fact, there is usually no difference at all in hydrogen-ion concentration between a 1 per cent solution of serum from a normal individual and a similar solution from a diseased one.

In making the phytopharmacologic test, we employed a special apparatus containing twenty-three parallel rows of ten perforations for holding test tubes in an upright position. The ten tubes in the first row, and sometimes those in two or three such rows, are filled with control solutions consisting of equal parts of Shive saline solution and distilled water. Succeeding rows of ten tubes each are filled with 1 per cent solutions of various specimens of blood serum to be dissolved and tested in the plant physiologic saline solution. The *Lupinus albus* seedlings are then carefully selected, measured, and immersed in the solutions filling the upright tubes. For ordinary routine examinations an average of ten seedlings for each solution to be tested is usually sufficient for a single experiment. The test should be repeated on several consecutive days, however, until the phytotoxic indices differ from each other by not more than 5 per cent. Such repetition of the test, on two days at least, is necessary because occasional crops of *Lupinus albus* yield seedlings impervious to saline solutions of toxins. Such seedlings give a negative reading or an index considerably higher than that yielded by controls in normal blood serum. While it is true that specimens of serum obtained from patients with certain rare conditions (i.e., virus diseases, etc.) consistently give higher readings, such conclusions can be deduced only from several tests. In dealing with specimens of serum of patients suspected of having pemphigus, pernicious anemia, or some similar condition, negative or exceedingly high readings after a single examination should be regarded as "no takes" if in several repetitions of the test toxic effects are definitely exhibited.

The seeds employed for the phytopharmacologic test by the Macht-Livingston school of plant physiologists are those of *Lupinus albus*, large variety. The dry seeds, approximately 10 mm. in diameter, are obtained from Italy, where this variety of *Lupinus albus* is widely cultivated. The test can be made with the small varieties of *Lupinus albus*, approximately 6 mm. in diameter, which are grown in this country, but the percentage of germination and uniformity of size of these seeds are much less than those of the French or Italian varieties. Seeds of the giant *Lupinus albus*, 13 to 15 mm. in diameter, may also be employed in this work and have the advantage of possessing a very definite line of demarcation between stem and root. However, Macht and his associates have derived the best results from the large Italian variety of *Lupinus albus* seeds, which are remarkably hardy and will germinate even when five years old, although the most satisfactory results are obtained with seeds that are one to two years old. *Lupinus albus* seeds are soaked overnight in tap water. The next day the swollen seeds are ready for planting. Finely ground sphagnum was found to be the most suitable medium for the problem in hand. The sphagnum should not only be ground to a fine powder but should also be impregnated with just the amount of water most suitable for optimum germination. Here the beginner in phytopharmacology encounters his first difficulty. If the moss contains too much moisture, the seeds will rot, fungi will develop, germination will be defective, and the seedlings will be diseased. If it contains too little water, the seedlings will suffer from malnutrition and lack of turgor and will exhibit a stunted growth. A great deal of experimentation has proved that the best results are obtained with sphagnum containing from 70 to 80 per cent of water by weight. Such a medium can be successfully prepared only after considerable practice. Sphagnum prepared for the germination of seedlings must be rinsed several times with hot water to free it from an excess of acid matter, which would interfere with proper development of the plants. It must also be well aerated before it is deposited in the glass jars in which the seeds are to be planted. Swollen seeds, which have been soaked overnight, are planted closely together with the hili pointing down. In this way a large number of seedlings can be raised in a comparatively small area. A light layer of the moss is then sifted over the seeds, and the covered jar is allowed to stand in the dark for several days at room temperature. When the weather is mild, the seedlings are generally of convenient length for testing on the third day after planting. In winter, however, germination progresses more slowly even at room temperature, and the seedlings are not long enough until the fourth day after planting.

The most suitable seedlings for the phytopharmacologic test are those with roots measuring from 35 to 60 mm. Shorter seedlings may be employed, but their growth is not usually so rapid as that of the longer ones. For this reason, in recent years we have been discarding those less than 30 mm. long. On the other hand, much longer seedlings exhibit curvatures which make them difficult to measure. Healthy seedlings are taken out of the moss; the adhering particles are removed by gentle brushing or rinsing, and the length of their

roots is measured with a millimeter rule. Here the beginner encounters a second difficulty. While the line of demarcation between root and stem is usually definite and easy to find with the naked eye, with or without the aid of some innocuous staining solution, consistently accurate readings can be made only after much practice and experience. After the seedling has been measured, it is immersed in a tube of either the control solution or the blood serum solution to be examined. In this way ten seedlings are selected for each solution tested, including controls. The roots of the seedlings in any given experiment must be measured both at the beginning and at the end of the test by the same person. A woman experimenter should not handle the plants at the time of catamenia. All experimenters should wash their hands carefully before touching the objects to be tested.

An important detail of technique is the *matching* of seedlings, i.e., the selection of seedlings of as nearly the same length as possible. Experiments with living plants are generally more reliable than those with living animals because of the vastly larger number of the former available and the physical and economic obstacles to such wholesale experimentation with higher animals. To insure maximum accuracy in performing the phytopharmacologic test, however, the seedlings selected should be of as nearly the same length as possible. Especially should the individual plants in corresponding positions in different rows be closely matched. This is accomplished by filling the first tube of each row with seedlings of as nearly the same length as possible, and then filling the second tube in each row with similarly matched seedlings and matching in the same way all the plants in corresponding tubes in the remainder of the rows.

When such a procedure is followed, deviations in readings due to differences in growth between seedlings which are either too long or too short at the outset of the experiment are reduced to a minimum, and a more reliable comparison can be made with regard to the effect of various solutions on the growth of the roots.

After all the seedlings have been measured and allotted to their respective tubes, the outfit containing them is placed in a refrigerator or a low-temperature incubator. The seedlings are allowed to grow there in the dark for twenty-four hours, at the end of which time they are measured again, and the average increment of growth in control and unknown solutions is determined. The average growth of roots in any given solution of blood sera divided by the average increment in the length of roots of control seedlings grown in Shive solution alone and maintained under exactly the same light and temperature, gives the so-called phytotoxic index of growth for that serum. Thus blood from normal persons yields a phytotoxic index ranging from 70 to 75 per cent, and serum from patients with pemphigus, a reading of 58 or 59 per cent.

Perhaps the most important single factor affecting phytopharmacologic testing of serum from patients with pemphigus is the temperature. It is absolutely

necessary that seedlings grown in various solutions of serum be maintained at the temperature most suitable for the test. In earlier publications one of us (D. I. M.) specified 20° C. as the temperature most suitable for such work. Later experience, however, has shown that the best results in testing specimens of serum from patients with pemphigus are obtained with seedlings grown at a considerably lower temperature. The blood from such patients changes rapidly when kept at room temperature. The pemphigus toxin decomposes even at from 18° to 20° C. in the presence of the slightly acid Shive solution. Furthermore, the stimulus to growth of seedlings kept at temperatures higher than 15° C. or 60° F. is so great that slight inhibitions produced by the presence of small quantities of toxin are masked. On the other hand, when the seedlings are maintained at temperatures below 15° C., the growth of all, even those in normal control solutions, is greatly retarded; as a consequence, the phytotoxic indices yielded by specimens of both toxic and normal blood are much lower than they should be. In other words, seedlings grown in solutions at temperatures lower than 15° C. give more toxic readings than those grown in similar solutions at 15° C. Of all the ecologic factors playing roles in phytopharmacologic work with blood, temperature is the most important. Undoubtedly some investigators attempting the test have obtained discordant results because they did not regard this all-important ecologic factor. It is obvious, moreover, that all specimens of blood sent for phytopharmacologic testing, especially those from patients in whom pemphigus is suspected, should be protected from light, and, preferably, kept on ice. One of us (D. I. M.) has repeatedly observed that the red corpuscles of blood affected by pemphigus undergo decomposition much more rapidly than normal erythrocytes and effect a purple hemolyzed fluid quite unsuitable for phytopharmacologic examination.

For investigative purposes in connection with new problems involving determination of small differences in growth of seedlings placed in control and unknown solutions, respectively, the phytopharmacologic test can be made more accurate by "proving" the seeds and seedlings. In such cases, after seeds have been soaked and germinated in sphagnum, and the root growth of a large number has been measured, they are placed in solutions containing equal parts of distilled water and Shive. The growth of all these seedlings and their roots under standard conditions, i.e., in the dark and at a temperature of 60° F., is then determined, and only those are selected for further phytopharmacologic work which show approximately the same rate of growth, a procedure which eliminates plants weaker than others and renders the readings much more reliable. It is unnecessary to reiterate that the findings obtained by the writers with large numbers of proved seedlings are at least as reliable as those derived from any zoopharmacologic test. By use of the phytopharmacologic method it is possible to determine the toxicity of many unknown substances with various controls for hundreds of biological test objects grown simultaneously, a feat very difficult to accomplish in zoopharmacologic experimentation and possible then only at the cost of much more time, money, and labor in a highly subsidized laboratory.

STANDARD DEVIATION, PROBABLE ERRORS, AND CRITICAL RATIOS

Illustrative Experiments

To illustrate the reliability and utility of the phytopharmacologic test of various pathologic blood sera, five sets of experiments are detailed and analyzed by modern statistical methods.^{17, 18} All five were carried on simultaneously under exactly the same conditions, and in each series there were 50 *Lupinus albus* seedlings with roots from 35 to 50 mm. in length. In the various solutions these seedlings were grown in the dark at 60° F. for twenty-four hours, at the end of which period the roots were measured again, and the phytotoxic indices were calculated. In Experiment A 50 control seedlings were grown in a mixture of equal parts of Shive and distilled water. Into the Shive solution, in which all the other series of seedlings were also grown, was introduced 1 per cent of (1) a normal human blood serum in Experiment B, of (2) a random specimen of blood from a patient tentatively diagnosed clinically as pemphigus in Experiment C, of (3) a serum obtained from a person after exposure to x-rays for nonmalignant disease in Experiment D, and of (4) a serum from a patient afflicted with schizophrenia of the hypokinetic type in Experiment E.

EXPERIMENT A

Increment in Root Length of 50 Controls in Shive Solution and Distilled Water (1:1)

17	16	14	12	13	13	13	18	12	14
23	15	13	15	16	9	13	14	13	14
15	12	15	9	11	13	11	11	13	13
12	10	15	12	13	15	12	14	16	11
14	9	10	11	13	13	13	11	12	15

$$\text{Average increment in growth} = 13.2 \text{ mm. } \sigma \text{ (Standard deviation)} = \sqrt{\frac{\sum D^2}{N}} = 2.0.$$

$$\text{P.E. (Probable error)} = \frac{0.6745 \times \sigma}{\sqrt{N}} = 0.19.$$

EXPERIMENT B

Increment in Root Length of 50 Seedlings in Shive Solution with 1 Per Cent Normal Blood Serum

9	8	8	10	7	7	8	9	9	10
14	22	8	9	10	10	10	7	11	10
9	10	13	12	8	9	11	11	9	10
11	9	13	7	10	12	11	10	11	12
9	12	9	7	7	12	12	7	10	9

$$\text{Average increment} = 9.5 \text{ mm. Phytotoxic index of normal blood serum} = 72 \text{ per cent.}$$

$$\sigma = \sqrt{\frac{\sum D^2}{N}} = 1.8$$

$$\text{P. E.} = \frac{0.6745 \times \sigma}{\sqrt{N}} = 0.22$$

$$\text{P. E. diff.} = \sqrt{\frac{\text{PE}^2}{\text{Shive}} + \frac{\text{PE}^2}{\text{Normal serum}}} = 0.254$$

Correlation of Shive Solution and Normal Serum Readings:

$$\text{Validity or critical ratio} = \frac{D}{\text{P. E. diff.}} = 14.5$$

EXPERIMENT C

Increment in Root Length of 50 Seedlings in Shive Solution With 1 Per Cent of Pemphigus Serum

6	5	5	6	5	5	7	5	5	5
6	5	2	5	7	4	6	4	4	3
7	5	7	4	5	4	7	7	6	5
6	4	4	5	5	3	5	4	6	5
6	2	3	8	7	8	6	4	5	7

Average increment = 5.2 mm.

Phytotoxic index of pemphigus serum = 40 per cent.

$$\sigma = \sqrt{\frac{\sum D^2}{N}} = 1.4$$

$$P. E. = \frac{0.6745 \times \sigma}{\sqrt{N}} = 0.124$$

$$P. E. \text{ diff.} = \sqrt{\frac{\overline{PE^2}}{\text{Normal serum}} + \frac{\overline{PE^2}}{\text{Pemphigus serum}}} = 0.216$$

Correlation of Normal Serum and Pemphigus Serum Readings:

$$\text{Validity or critical ratio} = \frac{D}{P. E. \text{ diff.}} = 19$$

EXPERIMENT D

Increment in Root Length of 50 Seedlings in 1 Per Cent Blood Serum, Obtained After X-ray Therapy

9	8	7	5	8	5	8	6	7	6
3	7	6	4	5	3	7	9	7	5
2	7	6	7	4	7	9	6	7	9
5	6	6	9	6	8	2	3	8	2
7	7	4	7	9	4	2	4	5	5

Average increment = 6.0 mm.

Phytotoxic index of x-ray blood serum = 46 per cent.

$$\sigma = \sqrt{\frac{\sum D^2}{N}} = 0.2$$

$$P. E. = \frac{0.6745 \times \sigma}{\sqrt{N}} = 0.019$$

$$P. E. \text{ diff.} = \sqrt{\frac{\overline{PE^2}}{\text{Normal serum}} + \frac{\overline{PE^2}}{\text{X-ray serum}}} = 0.17$$

Correlation of Normal Serum and X-ray Serum Readings:

$$\text{Validity or critical ratio} = \frac{D}{P. E. \text{ diff.}} = 23$$

EXPERIMENT E

Increment in Root Length of 50 Seedlings in 1 Per Cent Blood Serum. Obtained From a Psychotic Patient

9	6	7	2	7	4	9	8	8	5
6	3	7	5	5	5	2	7	9	5
6	7	6	3	6	2	9	3	7	5
6	7	6	7	7	9	6	5	5	4
6	6	9	4	7	5	8	5	7	6

Average increment = 6.0 mm.

Phytotoxic index of psychotic serum = 46 per cent.

$$\sigma = \sqrt{\frac{\sum D^2}{N}} = 0.183$$

$$P. E. = \frac{0.6745 \times \sigma}{\sqrt{N}} = 0.017$$

$$P. E. \text{ diff.} = \sqrt{\frac{PE^2}{\text{Normal serum}} + \frac{PE^2}{\text{Psychotic serum}}} = 0.17$$

Correlation of Normal Serum and Psychotic Serum Readings:

$$\text{Validity or critical ratio} = \frac{D}{P. E. \text{ diff.}} = 25$$

Since according to recognized mathematical criteria a critical ratio of 3.0 or more indicates that the difference between averages is a significant and not a chance difference, it will be seen that all the experiments analyzed to determine the standard deviation, probable error, and probable error of difference yield critical ratios of a much higher order and, therefore, reveal results of very high significance and reliability indeed.

ANALYSIS OF INTERGROUP VERSUS INTRAGROUP VARIATIONS BY FISHER'S METHOD

To further test the reliability of the foregoing data special analysis was made of the readings obtained for intergroup versus intragroup variations according to Fisher's well-known method.

Analysis of Variance.—Fundamentally analysis of variance is a test of significance. It is based on the solution of a number of problems of statistical distribution obtained by Fisher.¹⁹ The total variation in this experiment is separated into two parts, viz. (1) the variation attributed to irregularities in the results obtained in the given group, and (2) the variation attributed to the difference between the mean performances of the different groups. Once these two variabilities have been separated, they are compared to see whether or not there is a significant difference between them. In this way it can be determined whether the variation within the group is significantly greater or less than the variation between groups.

1. *Variation Within the Group.*—(a) The sums of the squares of the deviations from the individual means are computed. In this case they are:

Shive solution	=	200.960
Normal blood sera	=	181.090
Pemphigus blood sera	=	96.000

(b) The sums of these squares pooled are equal to 478.050. (c) The variance from this source is estimated by dividing the pooled sums of the squares by the degrees of freedom. The latter is the product of the number of group and one less the number of trials, i.e., $3 \times 49 = 147$. Thus the estimate of variance is $478.050/147 = 3.252$.

2. *Variation Between Groups.*—(a) The sum of the squares of the deviations of the sums from the mean of the sums is computed. Since the latter is the sum of the sums over 3 = 472.33, the step is as follows:

$$(496 - 472.33)^2 + (661 - 472.33)^2 + (260 - 472.33)^2 = 79.155$$

(b) This sum is divided by the number of trials per subject to reduce the sum of squares to a per item basis; thus $79.155/50 = 1.5831$. (c) The variance is estimated from this source by dividing the reduced sum of squares by the degrees of freedom of groups, i.e., by $n - 1 = 2$; thus $1.5831/2 = 0.79155$.

TABLE VII

COMPARISON OF READINGS OBTAINED WITH SEEDLINGS GROWN IN (1) SHIVE SOLUTION AND IN SOLUTIONS OF (2) NORMAL HUMAN BLOOD SERUM AND OF (3) PEMPHIGUS SERUM

SEEDLING	GROUP			TOTAL
	SHIVE	NORMAL BLOOD	PEMPHIGUS BLOOD	
1	17	9	6	
2	13	14	6	
3	15	9	7	
4	12	11	6	
5	14	9	6	
6	16	8	5	
7	15	12	5	
8	12	10	5	
9	10	9	4	
10	9	12	2	
11	14	8	5	
12	13	8	2	
13	15	13	7	
14	15	13	4	
15	10	9	3	
16	12	10	6	
17	15	9	5	
18	9	12	4	
19	12	7	5	
20	11	7	8	
21	13	7	5	
22	16	10	7	
23	11	8	5	
24	13	10	5	
25	13	7	7	
26	13	7	5	
27	9	10	4	
28	13	9	4	
29	15	12	3	
30	13	12	8	
31	13	8	7	
32	13	7	6	
33	11	11	7	
34	12	11	5	
35	13	12	6	
36	18	9	5	
37	14	7	4	
38	11	11	7	
39	14	10	4	
40	11	7	4	
41	12	9	5	
42	13	11	4	
43	13	9	6	
44	16	11	6	
45	12	10	5	
46	14	10	5	
47	14	10	3	
48	13	10	5	
49	11	12	5	
50	15	9	7	
Sums	661	496	260	1417
Means	13.22	9.92	5.20	28.34
Σ (deviations from means) ²	200.960	181.090	96.000	478.050

3. *Application of the F Test.*—This is that method described by Fisher²⁰ as "testing whether one estimate of variance derived from n_1 degrees of freedom is significantly greater than a second such estimate derived from n_2 degrees of freedom." A convenient adaptation is supplied by Mahalanobis²¹ and Snedecor.²² There are two steps in this test: the first is to form the ratio of the greater to the lesser variance, i.e., $791.55/3.252 = 243.5$; the second is to match this value in Fisher's table for the distribution of F, a table which shows how high the ratio must be to be barely significant and highly significant. It is entered with the degrees of freedom of the two variances forming the ratio. In evaluating the significance of the two degrees of freedom in this experiment, as outlined above, the figure 3.06 is regarded as barely significant and the figure 4.75 as highly significant. Thus the ratio of 243.5, which is far in excess of the higher ratio of 4.75, shows that the difference between the groups in this experiment is very significant indeed and greater than that within the groups.

COMPARISON OF READINGS OBTAINED WITH SEEDLINGS GROWN (1) IN SHIVE SOLUTION ALONE, (2) IN SHIVE SOLUTION WITH 1 PER CENT NORMAL BLOOD SERUM, AND (3) IN SHIVE SOLUTION WITH 1 PER CENT X-RAY BLOOD SERUM

	SHIVE SOLUTION	NORMAL BLOOD SERUM	X-RAY BLOOD SERUM
Sum	661	496	298
Mean	13.22	9.92	5.96
Σ deviations ²	200.96	181.09	203.89

Sum of deviations² = 585.94. Degrees of freedom = $3 \times 49 = 147$. Variance = $585.94/147 = 3.99$.

Sum of the sums = $661 + 496 + 298 = 1455$. Average = $1455/3 = 485$.

$$(661 - 485)^2 + (496 - 485)^2 + (298 - 485)^2 = 66066$$

$$66066/50 = 1321.32$$

$$1321.32/2 = 660.66$$

$$660.66/3.99 = 165.3$$

$$\text{Ratio of greater to lesser variance} = 165.3.$$

Thus it will again be seen that the intergroup difference is significantly greater than the intragroup difference.

COMPARISON OF READINGS OBTAINED WITH SEEDLINGS GROWN (1) IN SHIVE SOLUTION ALONE, (2) IN SHIVE SOLUTION WITH 1 PER CENT NORMAL BLOOD SERUM, AND (3) IN SHIVE SOLUTION WITH 1 PER CENT BLOOD SERUM FROM A PSYCHOTIC PATIENT

$$\text{Variance} = 3.79$$

$$\text{Sum of sums} = 661 + 496 + 298 = 1455$$

$$\text{Mean} = 1455/3 = 485$$

$$(661 - 485)^2 + (496 - 485)^2 + (298 - 485)^2 = 66066$$

$$66066/50 = 1321.32$$

$$1321.32/2 = 660.66$$

$$660.66/3.79 = 174$$

$$\text{Ratio of greater to lesser variance} = 174.$$

Again it will be observed that the intergroup difference is very significantly greater than the intragroup difference.

These detailed analyses of data obtained in typical experiments with control solutions in plant physiologic saline, on the one hand, and with normal serum and blood sera obtained from patients with pemphigus, x-ray therapy, and grave psychoses, on the other, leave no doubt as to the reliability of the

phytopharmacologic technique, as practiced in this laboratory, and the positive significance of the data obtained thereby. This, of course, applies to the phytopharmacologic test as employed by an experienced worker in the field. While our statistical analysis was made on long-range experiments, i.e., experiments in which 50 seedlings were employed for each solution studied, Macht and his co-workers have discovered in actual clinical practice that reliable data regarding the phytotoxic properties of blood sera are obtained by means of a smaller number of seedlings. The routine procedure in this laboratory is to employ ten healthy seedlings for each solution and to repeat the experiment on two or three successive days. If the phytotoxic indices derived from the successive experiments vary by no more than 5 per cent, the average figure obtained has been found to be very reliable as it almost invariably agrees with the final diagnosis of the blood picture made independently by the clinical practitioner and pathologist who forward the sample for pharmacologic study.

SUMMARY

1. The Macht technique for determination of phytotoxic reactions of blood sera is described in detail.

2. Tables presenting the phytotoxic indices obtained in phytopharmacologic examination of numerous toxic, nontoxic, and atoxic blood sera are offered for use as criteria in carrying on further work of this kind.

3. A biometric analysis of data obtained in typical experiments with normal human blood serum and blood sera from persons with pemphigus, x-ray therapy, and grave mental disease is made in detail to illustrate the reliability of the phytopharmacologic test and the significance of results secured by this means.

4. The various data adduced demonstrate the utility of the phytopharmacologic test or the application of plant physiology to investigation of clinical problems.

REFERENCES

1. Macht, D. I.: Contributions to Phytopharmacology or the Applications of Plant Physiology to Medical Problems, *Science* 71: 302, 1930.
2. Macht, D. I., and Livingston, M.: Effect of Cocaine on the Growth of *Lupinus albus*. A Contribution to "Phytopharmacology," *Proc. Soc. Exper. Biol. & Med.* 19: 235, 1922.
3. Macht, D. I., and Livingston, M.: Effect of Cocaine on the Growth of *Lupinus albus*. A Contribution to the Comparative Pharmacology of Animal and Plant Protoplasm, *J. Gen. Physiol.* 4: 573, 1922.
4. Macht, D. I., and Lubin, D.: A Phytopharmacological Study of Menstrual Toxin, *J. Pharmacol. & Exper. Therap.* 22: 413, 1924.
5. Macht, D. I., and Davis, M. E.: Experimental Studies, Old and New, on Menstrual Toxin, *J. Compar. Psychol.* 18: 113, 1934.
6. Macht, D. I.: A Phytopharmacological Study of Pernicious Anemia, *J. Pharmacol. & Exper. Therap.* 29: 461, 1926.
7. Macht, D. I.: Pernicious Anemia, an Experimental Contribution to the Etiology, Diagnosis and Treatment, *J. A. M. A.* 89: 753, 1927.
8. Macht, D. I.: Concerning the Nature and Distribution of Pernicious Anemia Toxin, *Festschrift of Professor Emil Bürgi*, p. 228, 1932.
9. Macht, D. I., and Paulson, M.: Phytopharmacology of Stomach Washings in Various Digestive Disorders and Pernicious Anemia, *J. LAB. & CLIN. MED.* 19: 155, 1933.
10. Pels, I. R., and Macht, D. I.: Phytopharmacological Examination of the Blood in Pemphigus and in Some Other Diseases of the Skin, *Arch. Dermat. & Syph.* 19: 640, 1929.

11. Pels, I. R., and Macht, D. I.: *Phytopharmacology of Pemphigus and Other Dermatoses, Additional Studies*, Arch. Dermat. & Syph. 23: 601, 1931.
12. Macht, D. I.: *Phytopharmacological Studies on the Blood of Trachoma Patients*, Folia ophth. orient. 1: 353, 1935.
13. Macht, D. I., and Gardner, R. E.: *Phytopharmacological Reactions of Normal, Toxic and Atoxic Sera*, Proc. Nat. Acad. Sc. 22: 384, 1936.
14. Macht, D. I.: *Effect of Roentgen Rays on the Toxicity of Blood*, Am. J. Roentgenol. 12: 709, 1939.
15. Macht, D. I., and Grumbein, M. L.: *Phytotoxic Reactions of Fresh, Frozen and Dried Blood Specimens*, Arch. internat. de pharmacodyn. et de therap. 60: 95, 1938.
16. Shive, J. W.: *A Study of Physical Balance in Nutrient Media*, Physiol. Researches 1: 327, 1915.
17. Garrett, H. E.: *Statistics in Psychology and Education*, Chicago, 1926, Longmans, Green & Co.
18. Treloar, A. E.: *Outline of Biometric Analysis*, Minneapolis, 1936, Burgess Publishing Co.
19. Fisher, R. A.: *Proceedings of International Mathematical Conference*, Toronto, 1924.
20. Fisher, R. A.: *Statistical Methods for Research Workers*, Edinburgh, 1935, Oliver & Boyd.
21. Mahalanobis, P. C.: *Ind. J. Agri. Sc.* 2: 694, 1932.
22. Suedecor, G. W.: *Analysis of Variance and Covariance*, Ames, 1934, Collegiate Press, Inc.

CLINICAL AND EXPERIMENTAL

SULFAPYRIDINE IN EXPERIMENTAL BRUCELLOSIS*

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WITH the advent of sulfapyridine in the field of therapeutics, especially in the treatment of pneumonia, a disease in which it produces such remarkable beneficial results, it was perhaps to be expected that it would be tried in the treatment of many other diseases, and to be hoped that it would show the same remarkable curative value in some of them as in pneumonia. Likewise, its ability to prevent and cure pneumococcal infections in experimental animals has been demonstrated many times by many investigators. Little has appeared in the literature so far reporting experimental results with this drug in the treatment of *Brucella* infections. On the contrary, many reports of the results of experimental work with regard to treating these infections with sulfanilamide are available. Many investigators have also reported various degrees of satisfactory results in the treatment of human brucellosis with sulfanilamide and its derivatives. Since the two drugs, sulfanilamide and sulfapyridine, are so closely related chemically, it is reasonable to expect them to show certain similarities in their therapeutic properties and that sulfapyridine might show even a better result in the treatment of these diseases.

Chinn¹ in a recent article reports that sulfanilamide has both preventive and curative effect on guinea pigs inoculated with *Br. abortus*, *Br. melitensis*, and *Br. suis*. Menefee and Poston² also report good results in the treatment of *Br. melitensis* var. *melitensis*, *abortus*, and *suis* with sulfanilamide.

To our knowledge no previous report on work with sulfapyridine in experimental *Brucella* infections of guinea pigs has been published.

The present report deals with results that were obtained with a group of guinea pigs after their experimental infection with the three *Brucella* organisms named above, as well as with the in vitro tests using the same organisms.

METHOD

The cultures used in this work were kindly supplied by Dr. I. F. Huddleson,³ of the Michigan State College of Agriculture. We found that these organisms grew well on meat extract agar, but resulted in a more abundant growth on beef liver infusion agar. Because they did not require increased carbon dioxide tension for growth, we did not use this method in any of our cultures.

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IN VIVO TESTS

We injected intraperitoneally 56 male guinea pigs of about the same age and size and divided them into three groups. The dose of organisms given each animal was one-half of a beef liver infusion agar slant suspended in normal saline solution. Normal saline solution slightly in excess of 2 c.c. was placed in the culture tube, and the organisms were stirred into an even suspension. One cubic centimeter was injected; therefore, one culture made two doses. Of the group of 56 guinea pigs, 16 were injected with *Br. melitensis*, 16 with *Br. abortus*, and 24 with *Br. suis*. These groups were subdivided into groups as follows: four, six, two, and four for each of the *Br. melitensis* and *Br. abortus*; and five, nine, four, and six for the *Br. suis*. The groups of four, four, and five in the case of each organism were given 100 mg. of sulfapyridine daily, orally, beginning one and one-half hours after the injection. The groups six, six, and nine in each organism were given 200 mg. of the drug by mouth daily beginning six days after the injection. The groups two, two, and four were given 100 mg. of sulfapyridine subcutaneously suspended in distilled water. In the groups four, four, and six no drug was given, and these animals served as controls. The drug was given once daily in a single dose in all instances by mouth, except as indicated above. The treatment was terminated at the end of three weeks in all groups. During the three weeks' period of treatment 15 animals died; therefore, the autopsy report contained 41 animals. The oral administration of the drug was accomplished with ease by giving a broken piece of the tablet rather than the powder. The animals receiving the drug beginning one and one-half hours after the injection were for the purpose of determining its preventive value, while from the group in which the drug was begun six days after injection we hoped to determine its curative effect. At the end of the treatment period all the animals were killed except half of the groups six, six, and nine. These were kept living for twenty-one days longer to determine by comparison with the other members of this group whether there occurred a flare-up of any hidden or latent infection. During the period of treatment all animals looked fairly normal except for a small per cent that appeared sick, some of which died. The greatest mortality rate occurred in the *Br. suis* group.

At autopsy the abdominal organs were noted for obvious pathology. No sections for microscopic study were made. In some of the animals gross evidence of disease was obvious in the form of small abscesslike lesions on the surface of the liver. There was also seen in some animals tough, exudative adhesions between the intestines and liver, liver and abdominal wall and intestines. The liver and spleen in all animals were weighed and were found in most cases not to exceed the normal weight. The average weight in the treated group of the spleen was 1.19 Gm. and of the liver 29.32 Gm. In the control group the average weight of the spleen was 1.12 Gm. and of the liver 30.46 Gm.

Beef liver infusion agar cultures were made from the sectioned liver and spleen from each animal. In a few instances cultures from the heart's blood were also made, but these were not completed. Since doing this work we attended a symposium on "Brucella Infection and Hodgkin's Disease" at Duke

University Medical School, participated in by Forbus, Poston, Gunter, and Wise, who report that the primary seat of involvement in experimental guinea pig infections with these organisms is found in the lymph glands. In our animals we did not notice any lymphadenopathy.

The cultures were examined after one week's incubation at 37° C. and evidence for positiveness was made by naked eye examination and Gram-stained smears. Table I summarizes these findings.

TABLE I

IDENTIFICATION MARK OF ANIMALS	SUIS			MELITENSIS			ABORTUS		
	LIVER	SPLEEN	HEART'S BLOOD	LIVER	SPLEEN	HEART'S BLOOD	LIVER	SPLEEN	HEART'S BLOOD
Blue (100 mg.)	-	-	-	-	+	-	-	+	-
	-	-	-	-	-	-	-	+	-
	-	-	-	-	-	-	-	-	-
Red (200 mg.)	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-
Control (No drug)	-	-	+	-	-	+	+	-	-
	-	+	-	+	-	-	-	-	-
	-	+	-	+	-	-	-	+	-
	-	-	-	-	+	-	-	+	-
Purple (100 mg. subcutaneously)	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-
"HOLD OVERS"									
Red (200 mg.)	+	+	+	-	-	-	-	+	+

*Each check indicates negative or positive culture findings and also each check under liver column represents one guinea pig.

IN VITRO TESTS

In order to determine the bactericidal (lethal) effect of sulfapyridine when mixed in broth cultures with the organism we followed this technique:

A forty-eight-hour beef liver infusion broth culture was prepared and a portion of this diluted 1:100. One-tenth cubic centimeter of this dilution was placed in each of five flasks containing 100 c.c. of beef liver infusion broth. To the first of these 100 mg. of sulfapyridine were added, giving a concentration of the drug of 1:1,000. A sterile 100 mg. per cent solution of sulfapyridine was prepared and to the second flask 10 c.c. were added, giving a drug concentration of 1:10,000; to the third flask 1 c.c. was added, giving a concentration of 1:100,000; to the fourth flask 0.1 c.c. was added, giving a drug concentration of 1:1,000,000; no drug was added to the fifth flask which served as a control. Immediately after mixing the organisms and drug, pour plates were made, using 0.1 c.c. of the drug-organism mixture and beef liver infusion agar as the pour medium. This was repeated at intervals of ten minutes: twenty-four, forty-eight, and seventy-two hours. After seven days' incubation of the pour plates counts were made with the results shown in Table II. The counts showed that the drug in 1:1,000 and 1:10,000 concentrations had definite or complete bactericidal power against *Br. melitensis* and *Br. suis*, but these

concentrations showed only definite bactericidal or bacteristatic effect against *Br. abortus*. In the 1:100,000 and 1:1,000,000 drug concentrations some bactericidal effect was noted against all organisms.

TABLE 11

PERIOD OF INCUBATION	NO DRUG	SULFAPYRIDINE 1:1,000	SULFAPYRIDINE 1:10,000	SULFAPYRIDINE 1:100,000	SULFAPYRIDINE 1:1,000,000
<i>Br. suis</i>					
Immediate*					
Count	0	10	10	1,100	180
10 min.	1,310	0	0	1,330	670
24 hr.	508,880	0	0	6,350	255,889
48 hr.	11,456,200	0	0	150,821	11,456,200
72 hr.	61,858,080	0	0	3,196,000	50,103,033
<i>Br. abortus</i>					
Immediate					
Count	670	3,380	2,750	3,190	2,810
10 min.	2,840	3,510	3,670	2,880	3,650
24 hr.	41,226,130	14,630	325,870	458,200	14,897,480
48 hr.	17,938,840	282,830	221,460	2,674,780	233,550
72 hr.	25,774,200	521,210	1,208,420	19,639,940	15,464,520
<i>Br. melitensis</i>					
Immediate					
Count	0	0	0	0	0
10 min.	20	0	0	0	0
24 hr.	180	0	380	700	122,180
48 hr.	5,487,040	0	910	1,179,880	17,772,640
72 hr.	19,333,190	0	1,000	9,330,260	28,743,030

*These figures are actual plate counts of bacteria per 1 c.c. of the different cultures.

CONCLUSIONS

We conclude from the results of this work that sulfapyridine in treating experimental brucellosis in the method employed in these experiments showed only a slight preventive or curative effect. However, we do think that it is significant that in the group of animals receiving 200 mg. there was not a single animal showing pathologic evidence or a positive culture, except in the group that was held over for quiescent infection determination. A glance at Table I will show that two groups of these were positive. This would seem to indicate that the infection was being held under control by the drug and underwent a flare-up after it was discontinued. We are aware of the fact that sulfapyridine, as given in this experiment, was not administered as frequently as is desired, yet the size of the largest dose when based on body weight, which is the usual way of computing dosage, is about eight to ten times larger than the average size for human beings, and this might in some way compensate for less frequent administration.

Armstrong and Muirhead⁴ recently reported that in a large series of guinea pigs 100 mg. of sulfapyridine orally every four hours maintained blood drug concentration at about the 7 mg. per cent level. Further, they report that 50 mg. orally every four hours did not keep the level up to what is generally considered to be optimum, while 200 mg. every four hours kept the level above the optimum of about 7 mg. per cent. On the basis of this work, we have reason to believe that after the drug was given in our animals the blood concentration reached about a 6 mg. per cent to a 15 mg. per cent level and then gradually fell before the next dose.

In our in vivo tests the drug seemed to show best results in *Br. suis* infections and poorest results in *Br. abortus*. In the animals held over for twenty-one days longer without treatment for evidence of latent or hidden infection, we found heavy infections in both *Br. suis* and *Br. abortus* animals and negative pathologic and cultural evidence with *Br. melitensis* animals.

The in vitro tests showed that in the 1:1,000 and 1:10,000 concentrations against *Br. suis* the drug resulted in complete destruction of the organism. In the 1:100,000 concentration against this organism definite bactericidal effect was noted, while the 1:1,000,000 dilution showed only feeble destructive power. These findings are in agreement with the in vivo findings, since it was in *Br. suis* infection that best results were noted. Against *Br. melitensis* the 1:1,000 drug concentration showed complete bactericidal power; the 1:10,000 nearly complete killing power; the 1:100,000 concentration definite destructive power; and the 1:1,000,000 concentration showed no killing power. The effect of the drug against *Br. abortus* was different when compared with the other two organisms. This organism was far more resistant; the 1:1,000 concentration showed a definite bactericidal effect only and the remaining dilutions showed less effect as the drug concentrations were reduced. In other words, the drug had far less killing power against *Br. abortus* when compared with its killing power against *Br. suis* and *Br. melitensis*. Therefore, it appears that in the therapy of these infections the drug is more potent against *Br. suis* infection, less potent against *Br. melitensis*, and least potent against *Br. abortus*.

SUMMARY

1. Three groups of normal healthy guinea pigs were each injected with *Br. suis*, *Br. melitensis*, and *Br. abortus*.

2. The dose of organisms used was one-half of a beef liver infusion agar slant culture.

3. Each main group of injected animals was divided into four subgroups and each subgroup was treated as follows: one subgroup was given 100 mg. of sulfapyridine daily by mouth, beginning one and one-half hours after injection; one was given 200 mg. of the drug daily by mouth, beginning six days after injection; one was given 100 mg. of the drug subcutaneously, beginning six days after injection; and one was not given any drug and served as a control group.

4. All treatment was terminated twenty-one days after injection.

5. At the end of this treatment period all animals were autopsied, except half of each of the groups receiving 200 mg. These were held for twenty-one days longer to determine whether a flare-up of the infection occurred during the no drug period.

6. The drug used for the in vitro tests was a 100 mg. per cent solution in distilled water. This was suggested by Dr. Robert Hoyt,⁵ of the University of Minnesota, who reported that he found a 100 mg. per cent solution satisfactory for general use.

7. The medium used throughout was beef liver infusion broth and agar. This medium was selected because it seems to possess certain nutritional substances promoting growth of these organisms.

8. The cultures used in these experiments were obtained from Dr. Huddleson, of Michigan State College.

9. We feel that the drug in the method used does not control or cure *Brucella* infections in guinea pigs, in spite of the fact that based on body weight the amount given was far greater than the usual (80 grains a day) therapeutic amounts given human beings. We believe that the drug has curative value against at least two of the types of *Brucella* infection (*Br. suis* and *Br. melitensis*) when given frequently enough to maintain a constant blood level.

REFERENCES

1. Chinn, B. D.: The Use of Sulfanilamide in Experimental Brucellosis, *J. Infect. Dis.* 64: 78, 1939.
2. Menefee, E. E., Jr., and Poston, M. A.: Effects of Sulfanilamide on *Brucella melitensis* var. *melitensis*, abortus and *suis*, *J. Bact.* 37: 269, 1939.
3. Huddleson, I. F.: Personal communication.
4. Armstrong, A. R., and Muirhead, D. R.: Sulfapyridine in Blood of Guinea Pigs After Oral Administration, *J. LAB. & CLIN. MED.* 24: 1281, 1939.
5. Hoyt, Robert: Personal communication.

CHANGES IN THE SEDIMENTATION RATE AND NONFILAMENT-FILAMENT RATIO FOLLOWING CLINICAL IMPROVEMENT IN PATIENTS WITH LOW-GRADE CHRONIC ILLNESS

A STATISTICAL ANALYSIS OF 323 CASES

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IN A STUDY of patients with low-grade chronic illness, in which chronic infection appeared to play an important part,¹ it was reported that (a) practically all patients showed an increased nonfilament-filament ratio; (b) more than 80 per cent showed an increased sedimentation rate; (c) the nonfilament-filament ratio and the sedimentation rate were higher in patients with severe symptoms than in those with moderate or mild symptoms; and (d) in acute exacerbations the nonfilament-filament ratio was increased relatively more than was the sedimentation rate. The present study was made to determine the character and extent of the changes in the nonfilament-filament ratio and the sedimentation rate which were associated with clinical improvement or recovery in a similar group of patients.

CLINICAL DATA

Studies of the blood were made on 323 patients with chronic or frequently recurring symptoms, many of which were probably secondary to low-grade chronic infection. Patients were grouped according to severity of illness, as having mild or recurrent, moderately severe, or severe symptoms. An additional group included patients with more or less acutely increased symptoms. Detailed analysis of a similar series of patients as to age, sex, and clinical diagnosis have been reported.¹

Following definite clinical improvement, whether spontaneous or following treatment, the blood studies were repeated. Improved patients were grouped as symptom free, or as having mild or moderate symptoms. Patients were also grouped according to the degree of improvement, from mild to symptom free, from moderate to mild, or from severe to moderate being considered as one stage; from moderate to symptom free or from severe to mild as two stages; and from severe to symptom free as three stages of improvement. The group of patients with acutely exacerbated symptoms was not included in this analysis because the nonfilament-filament ratio and the sedimentation rate did not conform to relationships found in other groups.¹

METHODS

Sedimentation rate determinations and complete blood counts were made on all patients. Except for nonfilament-filament ratios, hematologic data will not be discussed because the changes were inconstant or infrequent. In making differential counts neutrophils in which two or more lobes were united only by a filament of chromatin were recorded as filamented cells, others were classified as nonfilamented. The ratio of nonfilamented to filamented neutrophils (referred to as nonfilament-filament ratio) was expressed in percentage, i.e., nonfilament-filament ratio = $\frac{\text{nonfilamented cells} \times 100}{\text{filamented cells}}$.

In determining the sedimentation rate oxalated blood was drawn up to the 200 mm. mark on a Westergren tube. At the end of one hour the height of the column of clear fluid was recorded in millimeters.¹

TABLE I

CHANGES IN THE SEDIMENTATION RATE AND NONFILAMENT-FILAMENT RATIO IN PATIENTS SHOWING CLINICAL IMPROVEMENT

FINDINGS	PATIENTS	RANGE	MEAN	STANDARD DEVIATION	STANDARD ERROR
<i>Original</i>					
Nonfilament-filament ratio	299	10-100	29.75	14.34	0.83
Sedimentation rate	200	2-99 mm.	21.15	18.05	1.275
<i>Following improvement</i>					
Nonfilament-filament ratio	299	4-24	10.26	5.18	0.3
Sedimentation rate	200	1-35 mm.	6.425	5.85	0.41

Table I shows a comparison of the sedimentation rates and nonfilament-filament ratios, both originally and after definite improvement. The mean nonfilament-filament ratio of 299 patients was 29.7 originally and 10.3 following definite improvement. Although some of the patients showed higher ratios after improvement than did others originally, the difference between the mean values before and after improvement was 18.3 times its standard error. The mean sedimentation rate of 200 patients was 21.15 mm. originally and 6.42 mm. following improvement. The difference between the two mean values was 11.4 times its standard error.

As noted previously, patients were classified according to the number of stages of improvement (Table II). The reduction in the mean nonfilament-filament ratio was 40.7 in patients with three stages of improvement, 22.4 in

those with two stages, and 12.0 in those with one stage. The mean sedimentation rate was reduced 35.7 mm. in those with three stages, 18.4 mm. in those with two stages, and 7.5 mm. in those with one stage of improvement.

The relationship between improvement in clinical and laboratory findings was less constant in patients with only one stage of improvement, two patients in this group showing slightly higher nonfilament-filament ratios after improvement, and seven showing either no change or a slight increase in the sedimentation rate.

The differences between the mean gains shown by the various groups were all statistically significant.

TABLE II

RELATIONSHIP BETWEEN CHANGES IN THE SEDIMENTATION RATE AND NONFILAMENT-FILAMENT RATIO AND THE DEGREE OF CLINICAL IMPROVEMENT

DEGREE OF IMPROVEMENT	PATIENTS	RANGE	CHANGE IN VALUES		
			MEAN	STANDARD DEVIATION	STANDARD ERROR
1 stage					
Nonfilament-filament ratio	137	+3 to -29	-11.08	4.97	0.425
Sedimentation rate	94	+3 to -29 mm.	- 7.81	6.94	0.715
2 stages					
Nonfilament-filament ratio	113	-1 to -49	-22.38	9.33	0.88
Sedimentation rate	69	0 to -69 mm.	-18.44	13.88	1.67
3 stages					
Nonfilament-filament ratio	39	-17 to -75	-40.69	12.18	1.95
Sedimentation rate	26	- 5 to -94	-35.69	22.1	4.84

CONCLUSIONS

Clinical improvement in patients with low-grade chronic illness is accompanied by statistically significant improvement in the sedimentation rate and nonfilament-filament ratio.

The degree of improvement in the sedimentation rate and non-filament-filament ratio varies significantly with the degree of clinical improvement.

REFERENCE

1. Stiles, M. H.: Sedimentation Rate and Nonfilament-Filament Ratio in Low Grade Chronic Illness, Arch. Int. Med. 63: 664, 1939.
113 W. CHESTNUT HILL AVE.

BRONCHIECTASIS ASSOCIATED WITH MONILIA SIMULATING PULMONARY TUBERCULOSIS*

A CLINICAL-PATHOLOGIC STUDY

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BRONCHIECTASIS, because of the problems relative to etiology, prognosis, pathology, and treatment, has been a subject of interest to medical men from the time of its first description by Laënnec to the present day. Regarded as a comparatively rare disease when only autopsy material was available, the modern use of radio-opaque substance has shown the condition to be more prevalent than formerly believed, and both medical and surgical interest has correspondingly increased. Without the use of such radio-opaque substances the diagnosis is not easy, and its chronicity, complications, and verisimilitude to other pulmonary diseases has frequently led to errors in diagnosis. Infection of the bronchiectatic dilations by nearly all the microorganisms found in pulmonary disease is almost a logical sequence of events and has been the subject of several papers and discussions by many men. The following is submitted as a peculiar infection in a well-defined case of bronchiectasis, erroneously diagnosed as pulmonary tuberculosis.

E. W., a white, unmarried woman of 30 years, entered the White Haven Sanatorium on April 4, 1938, with a diagnosis of pulmonary tuberculosis in the service of one of us (E. W. B.). Except for the fact that her father had died of carcinoma of the stomach in 1912, the family history was negative. Her past history included an attack of influenza in 1919, two attacks of pleurisy without effusion and bronchitis previous to 1936, and numerous colds. There was no history of whooping cough or of any contact with tuberculosis. The present illness began in April, 1936, with a pulmonary hemorrhage of 8 ounces. After approximately three months' rest at home, the patient went to a private sanatorium from July to October, 1936, but returned to work as a stenographer on November 1 of that same year. She did well until March 7, 1938, when she had a second severe and protracted hemorrhage of approximately 24 ounces. A few days afterwards x-rays of the chest were taken and sputum tests were made. Although no tubercle bacilli were found in the sputum, the patient was diagnosed as having pulmonary tuberculosis on the basis of the history, clinical evidence, and x-ray findings—a perfectly reasonable assumption. Her account of the cough of which she complained was interesting. She was quite definite in her statement that the many "colds" with which she was afflicted began in April, 1935, a year before her first hemorrhage. She had coughed ever since that date; her sputum was an odorless, yellowish green fluid, and varied in amount from 1 to 4 ounces daily. Despite many tests, no tubercle bacilli were demonstrated.

*From the Medical Department of the White Haven Sanatorium, White Haven, Pa.
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Upon admission the patient was a rather sickly appearing pale woman. The right side of her chest, somewhat smaller than the left, did not expand freely. The quality of the percussion note increased from impairment in the upper part to distinct dullness at the base where there was a suggestion of a pleural rub. The whispered voice was increased throughout the entire right side of the chest; the breath sounds were bronchial in character and coarse râles, especially at the base, were present. The left side of the chest was a trifle full and expansion was good; the percussion note was impaired in the upper part where the whispered voice was somewhat increased and the breath sounds were bronchovesicular in character. Otherwise the left side of the chest was negative. The cardiac point of maximum impulse was found in the fifth intercostal space inside the midclavicular line. No cardiac murmurs were heard. The fingers were not clubbed, nor were the nails curved. The blood pressure upon admission was 116/80, the red blood cell count was 4,400,000, the white blood cell count was 10,200, of which 68 per cent were polymorphonuclear cells and 30 per cent lymphocytes. The hemoglobin was 80 per cent and the Wassermann test was negative. Repeated sputum examinations were, and continued to be, negative for Koch's bacillus. The urinalysis was negative. Examination by Dr. Joseph C. Donnelly, of Philadelphia, revealed infection of the left maxillary sinus. The x-ray report of April 15, 1938, read as follows:

"The left lung is essentially normal. The shadows over the lower portion of the right lung are evenly increased in density, and there is a distinct atrophy of the right side of the chest indicative of a bronchial occlusion. The etiological factor is not clear, but if the sputum be negative, I would be suspicious of a benign growth. A pneumonography and a bronchoscopic examination may throw some further light on the condition." (Fig. 1.)

The last sentence in this report strengthened our determination to use some radioactive substance to determine the presence or absence of a condition we had previously suspected from a study of the history, clinical findings, and x-ray films on the case. Lipiodol was introduced into the trachea by the intertracheothyroid route on May 16, 1938, and the x-ray report read as follows:

"The iodized oil has entered the bronchial branches of the right lower and I believe the right middle lobe. It has collected in pools in cylindrical dilatations along the bronchi. The changes are those of a bronchiectasis involving particularly the lower lobe and I suspect there is also some involvement of the middle lobe." (Fig. 2 and Fig. 3.)

The patient's temperature, 101° F. upon admission to the White Haven Sanatorium, became normal under the influence of bed rest. Her pulse, 108 when the patient was first seen, also became normal under a like treatment. She weighed 94 pounds when she first came to the White Haven Sanatorium and gained 25 pounds in the next six months (119 pounds).

Even before the diagnosis of bronchiectasis was thus made, a number of yeastlike cells were discovered in the sputum during the continuous search for the tubercle bacilli. A culture medium of glucose, powdered raw potato, peptone, and agar was inoculated with the sputum and cultivation was carried on at room temperature. An organism which closely resembled *Monilia albicans* (Castellani) was identified by morphologic, structural, and cultural characteristics. The cells, either round or somewhat oval in shape, varied in size; the mycelium was septate and conidia were formed both by budding near the joints and abstriction at the ends of the hyphae. The culture characteristics were tested in a strained medium made up of peptone, powdered raw potato, and water; to this were added in different fermentation tubes dextrose, maltose, lactose, galactose, levulose, dextrin, and monnite—a separate sugar to each tube; aqueous acid fuchsin (0.5 per cent) was used to

determine the formation of acid during the growth of the fungus in these tubes during the next two or three weeks at room temperature. Gas and acid formation were observed in the tubes containing dextrose, levulose, maltose, and galactose in three successive cultures during the next ten weeks. The organism was identified as *Monilia albicans* (Castellani).

Our inability to discover any evidence of thrush in the mouth or pharynx was confirmed by Dr. M. J. Hess (D.D.S.) of White Haven, who cleared the mouth of all objectionable or suspicious elements. The gums were thoroughly healed before work was begun upon the sputum, and the patient was instructed to use a strong mouth wash before expectorating—a precept which we are certain she rigidly followed.

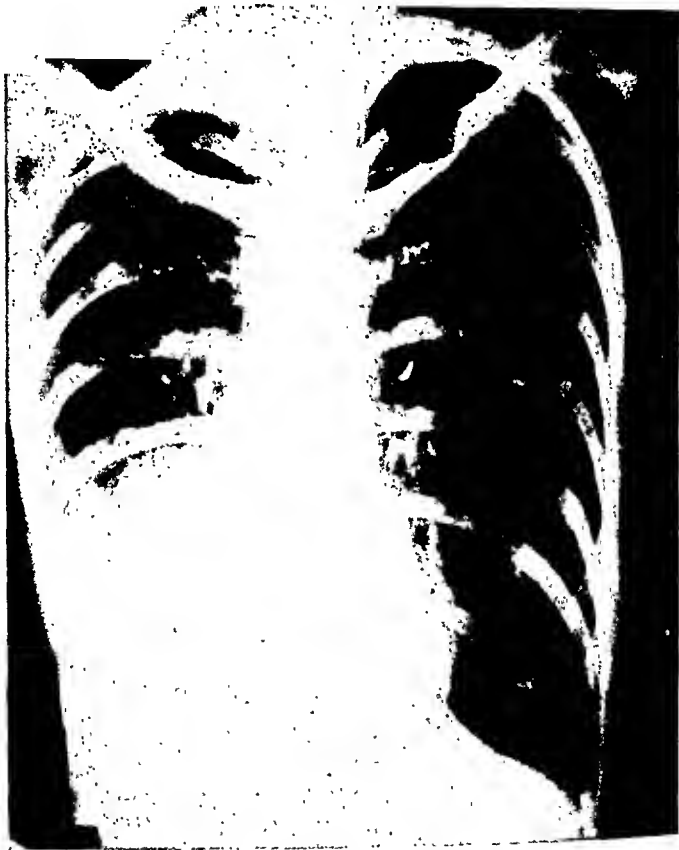


Fig. 1.

COMMENTS

Because of the generally accepted opinion of medical men that the disease is one seen only by those engaged in tropical medicine—and possibly also because of the little space devoted to the subject in even the best textbooks on diseases of the lung—fungus infection of the respiratory tract has received little attention to the present time from the medical profession of the temperate zone. A growing literature within the past fifteen years, however, has aroused interest in the disease, and has placed among diagnostic possibilities a condition that may not only simulate especially the more chronic diseases of the lung, but may affect the prognosis when seen as a complication and may even cause death.



Fig. 2.



Fig. 3.

Classification of the microorganisms included in the subject of mycology is in a state of almost hopeless confusion. Almost any one of the authorities seems to mention the classification proposed by another authority only to differ from it and to propose his own classification in its stead. One authority, indeed, goes so far as to suggest the scrapping of all classifications thus far proposed and to include all fungi under the general heading of blastomyces. Nor do they agree as to methods. Castellani, for example, although admitting the occasional failure of the procedure, lays stress upon the ability of the microorganisms to ferment the various sugars and to produce acid in the different solutions as a means of identification. Dodge, on the other hand, scouts the idea and seems to rely solely upon the morphology of the microorganism in question. Even the biological relationship between monilia, oidia, torula, and yeasts is not a settled thing among various authors, although almost without exception they classify the monilia among fungi imperfecta.

Irrespective of classification, however, all authorities agree that fungus infection of the lung is possible and divide the cases into three types. In the mild type of infection the general condition of the patient is not much impaired. There is little or no fever; the sputum is generally scanty and mucopurulent in character; a few râles may be heard but usually examination of the chest is negative. In the intermediate type there is evidence of a mild catarrhal bronchitis; a slight fever is usually found; the cough, differing in character in different patients, is usually paroxysmal and, as a rule, is most severe in the morning and the evening. Dyspnea is not infrequently one of the symptoms. In the severe type the patient loses weight, the fever is hectic, the sputum is rather profuse, and at times hemorrhagic; night sweats and a marked anemia are very often found. Chest examination reveals areas of dullness, fine râles, and evidences of pleuritis; in short, the picture of the severe type closely resembles that of pulmonary tuberculosis, for which it may be readily mistaken. The prognosis naturally varies with the virulence of the infection. The mild type may clear up spontaneously or after several weeks or months may develop into the intermediate or the more severe type; it usually yields to treatment. The intermediate type is more stubborn, and the response to treatment is often slow. The therapy must be continued, as such cases may readily develop into the third type. The severe type is often difficult to combat, and cases of death due to mycotic infection of the lung are not wanting (Stovall and Greeley, Warr).

The x-ray film in this disease also closely resembles that of pulmonary tuberculosis. There may be peribronchial thickening, nodules, infiltration, pleural thickening, areas of consolidation, and even fibrosis shown in the film (Bakst, Hazard, and Foley). The apices usually remain clear even in the most advanced cases, the disease being essentially a basal one (Balog and Grossi). Little is known of the pathologic findings in man, as few autopsies have been reported. In the case cited (Warr) both right and left lungs were adherent to the chest wall; there were numerous cavities on each side, and the lower lungs were consolidated and easily torn. The cut surfaces were glistening, shiny, and translucent. Both lungs were greatly reduced in size with little air-bearing space in either. Potassium iodide seems of value in the treatment of the disease,

especially of the first and second types. Castellani advocates the administration of 15 grains of potassium iodide in milk or water three or four times a day over a period of three to four weeks. Other medication has also been lauded. Balog and Grossi favor the use of insulin, Stovall and Greeley gave gentian violet intravenously. Thymol also has its advocates. Vaccine treatment has been proposed, and Kotkis, Wachowiak, and Fleisher, for example, reported complete disappearance of symptoms in one case and marked improvement in another. Balog and Grossi are of the opinion that vaccine treatment is the sole therapy capable of restoring the injured myocardium in moniliasis. Craik reports the successful use of alkalis, adrenalin, and potassium iodide. The roentgen ray as a therapeutic means has been used by Schmidt and Howe. Warr classes alkalis and iodide by mouth, and autogenous vaccines and ultraviolet rays as energy providers.

SUMMARY

A case of bronchiectasis in conjunction with *Monilia albicans* has been reported, together with a brief discussion on mycotic infection of the respiratory tract. We are of the opinion that mycotic infection of the respiratory tract is of more frequent occurrence than is generally believed, and that careful search of cases closely simulating pulmonary tuberculosis in the absence of tubercle bacilli in the sputum will reveal this condition, especially where the lesion is of the basal type.

In conclusion, may we say that we do not wish to leave the impression that we believe that *Monilia* was in any way responsible for the bronchiectasis, but rather that the bronchiectasis offered a very favorable soil for the growth of the fungus when once it had reached the lung. Another point worthy of note is that bronchiectasis and moniliasis, both pre-eminently basal affections of the lung, individually closely simulate basal pulmonary tuberculosis. The combination of the two diseases certainly more than doubles the resemblance. The fact that the specimens were not obtained directly with the bronchoscope need not militate against the results as all possible means were taken to eliminate oral contaminations. If this failure is a fault, then it is one shared by all cases we found in literature.

Comments by Dr. E. W. Bixby: The history of the case to date is also interesting. After it had been determined by all the usual tests (including animal inoculation) that the patient was not suffering from pulmonary tuberculosis, she was removed from the White Haven Sanatorium on November 19, 1938, to a hospital in Wilkes-Barre, where her accessory nasal sinuses were thoroughly drained. Ten bronchoscopic drainages were also done at the same hospital. She was then transferred to the Pennsylvania Hospital, Philadelphia, in the latter part of June, 1939, where Dr. John B. Flick removed the lower lobe of the right lung. Because of the many and dense adhesions present, the operation was a very prolonged and difficult task, but the patient withstood the operation well and made an uneventful recovery until August 15, 1939, at which time she developed an empyema. Closed drainage was instituted and a recent letter from Doctor Flick reports the patient in good condition.

REFERENCES

- Castellani, Aldo, and Chalmers, A. J.: *Manual of Tropical Medicine*, ed. 3, Baltimore, 1919, Williams & Wilkins.
Dodge, C. W.: *Medical Mycology*, St. Louis, 1935, The C. V. Mosby Co.

- Stovall, W. D., and Greeley, H. P.: *Bronchomycosis*, J. A. M. A. 91: 1346, 1928.
- Bakst, H. J., Hazard, J. B., and Foley, J. A.: *Pulmonary Moniliasis*, J. A. M. A. 102: 1208, 1934.
- Balog, J., and Grossi, Gino: *Clinical and Experimental Studies on Castellani's Pulmonary Moniliasis*, J. Trop. Med. 32: 253, 1929.
- Warr, O. S.: *Bronchomoniliasis*, Am. J. Int. Med. 5: 307, 1931.
- Kotkis, A. J., Wachowiak, M., and Fleisher, M. S.: *Relation of Monilia to Infections of Upper Air Passages*, Arch. Int. Med. 38: 217, 1926.
- Craik, R.: *Moniliasis and Tuberculosis*, Brit. M. J. 1: 682, 1929.
- Schmidt, J. M., and Howe, A. C.: *Treatment of Bronchomycosis With X-ray*, New York State J. Med. 25: 60, 1925.

PRIMARY MALIGNANT TUMORS OF THE SPLEEN WITH REPORT OF A CASE OF LYMPHOSARCOMA*

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THE infrequency of primary malignant tumors of the spleen affords sufficient reason for reporting single cases as they occur in the practice of individual surgeons, or as they are found on the autopsy table by pathologists. Examination of the literature shows that very few surgeons have removed more than one tumor of this kind; and in this connection it may be stated that the case here reported is the only one to be found in the records of the Jefferson Medical College Hospital.

In 1929 Tasker Howard,¹ of Brooklyn, found 115 cases recorded in the literature and added another case that had occurred in his own practice. Since the publication of Howard's paper, I have succeeded in finding 23 more evidently authentic cases²⁻²² which, with the one here reported, brings the total number of cases to 140.

Because of the rarity with which these cases occur, and also because of the complex structure of the spleen, classification of the tumors has been difficult. Growths called carcinoma have been reported, but the histologic structure of the spleen renders the development of a primary epithelial growth impossible, unless it is of the fetal inclusion type.

Various names have been applied to tumors of the connective tissue type, and even at the present time it would seem that a definite classification has not been firmly established. For instance, as late as 1935, Stevenin¹⁷ and his associates studied a tumor, which, they stated, could not be placed in any of the classifications previously made. Because of its resemblance to undifferentiated reticular sarcoma of bone marrow, they finally decided to call it undifferentiated reticular sarcoma.

Without reviewing the various classifications, it may be stated that a primary malignant neoplasm may take origin from the connective tissue in the splenic capsule; from the reticular stroma of the spleen; from the endothelial

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cells in the walls of the blood vessels; from the reticulo-endothelial cells lining the splenic sinuses; and from the lymphoid tissue surrounding the arterial vessels as an interrupted sheath, known as the splenic nodules or Malpighian corpuscles. The endothelial and lymphoid types are apparently the most common, the latter being termed lymphosarcoma. Vascular growths known as malignant hemangioma, hemangiosarcoma, and hemangio-endothelioma are also of relatively frequent occurrence.

The case that I am now reporting is that of a man, who at the time I operated upon him, January 5, 1928, was 58 years old. His history prior to the development of the splenic tumor is not without interest, in that it shows a susceptibility to disease of the reticulo-endothelial system. On two occasions, first in 1909 and again in 1921, enlarged lymph nodes were removed from the inguinal and cervical regions, respectively. In the interval of twelve years between the two operations, and also after the second operation, no evidence of enlarged lymph nodes was noticed in any part of the body.

Unfortunately, no record of microscopic examination of the inguinal nodes removed in 1909 was obtainable. Dr. Baxter L. Crawford, who examined the discrete cervical nodes removed in 1921, stated that the histologic picture was one of malignancy rather than of chronic inflammation. The possibility of lymphogranuloma was suggested, but it was definitely stated that the lesion was not typical.

Late in December, 1927, the patient consulted me again, complaining of digestive disturbance, weakness, and high abdominal pain. He stated that approximately six weeks before his visit, he noticed a swelling, which had increased rapidly in size, in the left part of the abdomen below the edge of the ribs. He also stated that he had lost several pounds in weight during the preceding month.

Examination revealed a large left-sided abdominal mass, distinctly nodular, and plainly splenic in origin. There was no fever, and blood examination, with the exception of a slight anemia of the secondary type, failed to show any abnormality. X-ray examination showed nothing significant, except the shadow of the splenic mass.

Splenectomy was performed on January 5, 1928, in the Jefferson Medical College Hospital. The tumorous spleen was very adherent to the diaphragm and had enlarged so much in the direction of the stomach that it had obliterated the space between the folds of the gastrosplenic omentum. It was, however, removed without any especial difficulty.

The pathologic report submitted by Dr. Baxter L. Crawford was as follows:

"Specimen consists of a spleen which measures 18 by 13 by 8 cm. and weighs 735 Gm. The external surface of the greater part of the spleen is nodular, and the nodules vary in size, and are grayish red in color in sharp contrast to the red color of the splenic tissue. These nodular areas are extremely soft and in some places seem to fluctuate. On section it is seen that the greater part of the splenic pulp has been replaced by nodules of soft gray, homogeneous tissue, which are discrete and sharply defined at the margins. They do not appear to have invaded the splenic capsule. In the larger nodules the tissue is soft, yellowish gray in color, and large areas show necrosis. The small nodules are sharply defined, pearly white in color and soft in consistency.

"Formalin and Zenker's fixations. Routine technique. Histology: Sections show the tumor masses to be composed of cells which vary somewhat in size and shape, but which for the most part are round with rather large, deeply staining nuclei and with a small amount of fibrous tissue stroma. There are numerous small blood vessels in this stroma. The margins of the tumor are sharply circumscribed, and although there is a condensation of the tissue, no definite capsular formation is observed.

"In the adjoining splenic tissue the follicles are numerous, large, and irregular in shape. Many possess large germinal centers. There seems to be a considerable proliferation of the cells of the follicles. The pulp of the spleen appears practically normal.



Fig. 1.—Photomicrograph of a section of the spleen ($\times 50$) showing edge of tumor; the tumor is above, the normal splenic tissue below and to the left. Observe also slight invasion of the spleen along the inferior portion of the section.

"There is no doubt that the lesion in the spleen is a malignant neoplasm, but there is some question as to the origin and type of the cell forming the growth. Because of the multiplicity of the tumors and the marked hyperplasia of the follicles, it is considered to be of lymphoid cell origin, even though in the tumor masses the cells are not identical as to the size and lymphoid type of cell.

"In the larger nodules there is extensive necrosis of the tissue.

"Sections were also stained to demonstrate the reticulum, but nothing unusual was shown in that structure.

"Diagnosis: Lymphosarcoma of the spleen" (Fig. 1).

The patient made a good postoperative recovery and went back to work within a few weeks. He remained in fairly good health until autumn. In November he began to complain of weakness, pain in the thorax, and some difficulty in urinating, and his gait became unsteady. These symptoms increased rapidly, and on December 30 he was read-

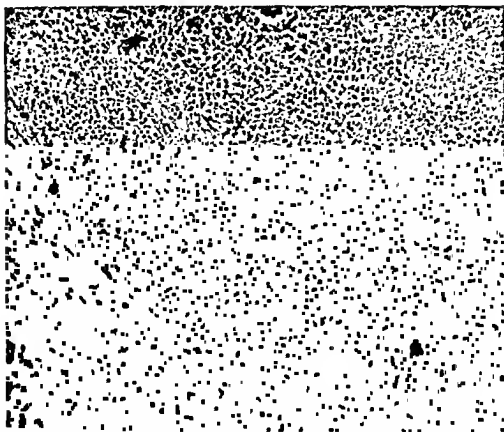


Fig 2.—Photomicrograph of a section of the liver ($\times 100$) showing invasion by the tumor; the tumor is above and to the right, the normal liver tissue below and to the left.

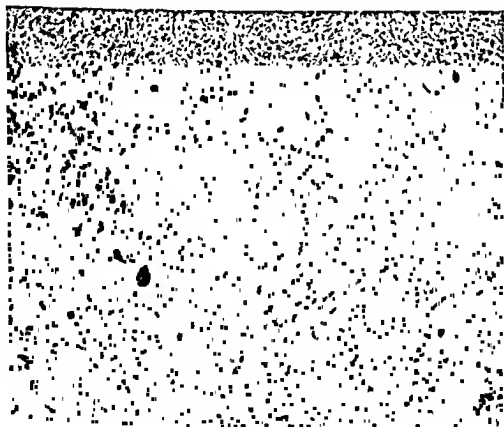


Fig. 3.—Photomicrograph of a section of the lung ($\times 100$) showing generalized invasion by the tumor.

mitted to the hospital. At that time he had complete retention of urine and had to be catheterized. By January 15, 1929, there was complete paralysis of the lower extremities and loss of sensation from the soles of the feet to the level of the umbilicus. The paralysis was accompanied by loss of control of both bladder and rectum and was soon followed by the development of gangrenous areas over the sacrum and on the lower extremities. Death occurred on February 18, 1929.

Autopsy was performed the same day by Dr. Crawford. Abridging his report, the following summary may be made: In the liver innumerable grayish nodules of variable

size and consistency were found (Fig. 2). There were also three similar nodules in the left lung (Fig. 3), and one in the cortex of the left kidney (Fig. 4). When the spinal cord was removed, a mass of firm grayish tissue was seen surrounding its lower thoracic and upper lumbar portions (Fig. 5).

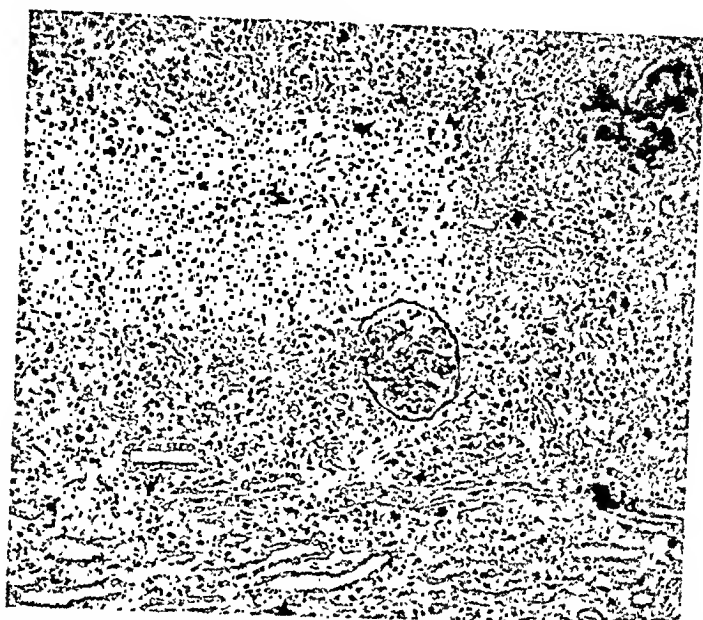


Fig. 4.—Photomicrograph of a section of the kidney ($\times 100$) showing invasion by the tumor, especially above and to the left.

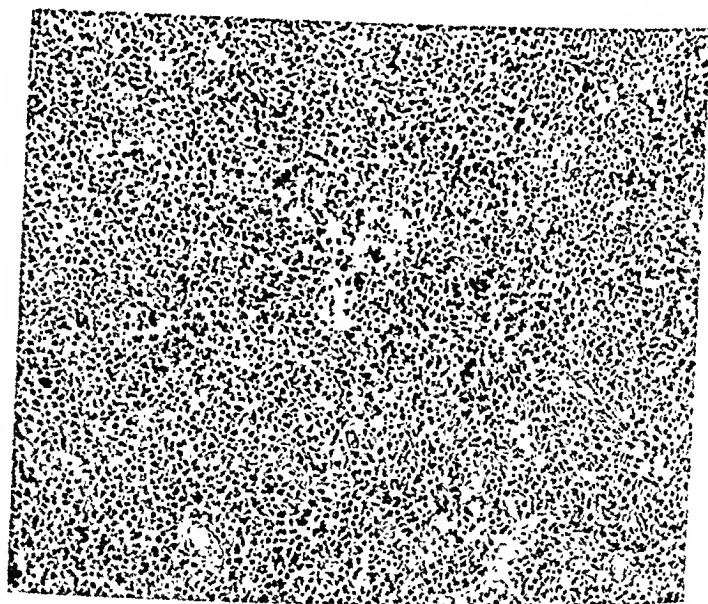


Fig. 5.—Photomicrograph of a section of the metastatic growth removed from the spinal cord ($\times 100$) showing its cellular structure.

The histologic structure of nodules removed from all three of these organs, and that of the neoplastic tissue dissected from the spinal cord, was quite similar to the histologic structure of the splenic tumor previously examined. Therefore, a diagnosis of multiple metastatic sarcoma was made.

It is noteworthy that there was no involvement of lymph nodes in any part of the body. A few of the mesenteric and retroperitoneal nodes were slightly enlarged, but microscopic examination showed only hyperplastic changes.

With regard to early symptomatology, it may be stated that malignant tumors of the spleen usually grow rapidly and that they are characterized by local pain, tenderness to pressure, and gastrointestinal disturbances. Very rapid loss of weight has occurred in some cases.

Changes in the blood are inconstant, but Howard,¹ Frank,¹³ and McNee,¹⁵ making independent observations, have called attention to the fact that in lymphosarcoma the picture may simulate that of pernicious anemia. In some cases, however, slight secondary anemia has been the only abnormality detected until late in the course of the disease; and even in some far-advanced cases the changes have been insignificant.

The growth may invade adjacent structures by direct extension and give rise to serious complications. Thus, fatal peritonitis has followed perforation of the colon caused by neoplastic invasion of that structure. The pleura has also been involved by extension of the growth through the diaphragm.

Metastases will affect the clinical picture according to the site in which they occur. For example, in the case of my patient, pressure upon the spinal cord produced a transverse myelitis, which accounted for the paralysis of the lower half of the body, with its accompanying urinary dysfunction and infection, and the development of gangrene over different areas of the paralyzed region.

From the surgeon's standpoint, malignant tumors of the spleen will require differentiation principally from benign tumors, cysts, and abscesses. Infarction without abscess formation should also be taken into consideration. It would seem that the majority of these tumors are nodular, but others, particularly some of the endotheliomas, are smooth. Absence of the normal notches along the anterior border of an enlarged spleen should arouse suspicion of tumor formation.

The prognosis is bad. In the 23 cases that I have collected from the literature, splenectomy was performed in 15. In two other instances the condition was found to be inoperable when the abdomen of the patient was opened. One of the patients died a few hours after operation; the other lived for four and one-half months. Of the nine who survived splenectomy, five are known to have died of metastases at periods varying from a few weeks to eighteen months after their discharge from the hospital. In three of the five remaining cases only a few months had elapsed between operation and publication of the case reports, so that a definite statement as to the ultimate result could not be made. The fourth patient, who had remained free from recurrence for nearly four years, developed a mass in the left flank, but it was questionable whether the lesion was metastatic sarcoma or primary carcinoma of the colon. The patient refused operation. The fifth patient was well five and one-half years after operation. The case was one of hemangio-endothelioma. Death occurred in the six cases in which operation was not undertaken.

Some pathologists believe lymphosarcoma to be part of a generalized malignant process, which will attack other structures in the reticulo-endothelial sys-

tem simultaneously with, or subsequently to, its beginning in the spleen. Furthermore, endothelial tumors have been followed by metastasis in a very short time after their removal. In a few cases, however, including both types of tumor, there has not been any reappearance of the disease for several years after extirpation of the spleen; consequently, splenectomy is advisable, provided that there are no signs of involvement of other organs when the patient first came under observation.

I am greatly indebted to Dr. Baxter L. Crawford and Dr. Carl J. Bucher, of the Pathological Department of the Jefferson Medical College and Hospital, for their cooperation in the study of the case herein reported.

REFERENCES

1. Howard, T.: Primary Sarcoma of Spleen (Reticulum Celled), Report of a Case, *J. LAB. & CLIN. MED.* 14: 1157, 1929.
2. Wright, A. W.: Primary Malignant Hemangioma of the Spleen With Multiple Liver Metastases, *Am. J. Path.* 4: 507, 1928.
3. Taylor, A. L.: Case of Primary Sarcoma of the Spleen, *Bristol Med.-Chir. J.* 46: 121, 1929.
4. Sabrazès, J., and Duperier, R.: Réticulo-endothélio sarcome de la rate, *Bull. de l'assoc. des Anatomistes* 18: 454, 1929.
5. Klein, F.: Primary Sarcoma of the Spleen, *Bratisl. lekár. listy.* 9: 47, 1929.
6. Kasabakas, D.: Ein Primäres Lymphoplastisches Sarkom der Milz, Inaugural Dissertation, Munich, 1929.
7. Wright, J. H., and Stevenson, E. M. K.: Case of Primary Sarcoma of Spleen, *Glasgow M. J.* 114: 1, 1930.
8. Dakenavicius, S.: Primary Sarcoma of Spleen, *Medicina, Kaunas* 11: 619, 1930.
9. Zeno, A., and Cid, J. M.: Hemangio-Endotheliome de la rate, *Ann. d'anat. path.* 7: 583, 1930.
10. Eearius, O.: Beitrag zur Chirurgie der Milztumoren, *Arch. f. klin. Chir.* 163: 55, 1930.
11. Sturgeon, C. T., and Ware, E. R.: Primary Lymphosarcoma of Spleen, *S. Clin. North America* 11: 1163, 1931.
12. Paine, C. G.: Malignant Tumor of the Spleen and Bone Marrow With Metastases in Liver, *J. Path. & Bact.* 34: 139, 1931.
13. Frank, L.: Sarcoma of Spleen, *Am. J. M. Sc.* 183: 77, 1932.
14. Caldwell, G. T.: Endothelioma of Spleen With Report of Case, *South. M. J.* 26: 120, 1933.
15. McNee, J. W.: Sarcoma of Spleen, *J. Path. & Bact.* 39: 83, 1934.
16. Wieden, L.: Ueber Milzextirpationen und Nachuntersuchungen an Milzextirpierten, *Mitt. a. d. Grenzgeb. d. Med. u. Chir.* 44: 13, 1935.
17. Stevenin, H., Bergeret, A., Albot, G., and Lelourdy, J.: Le reticulosarcoma de la rate, *Presse méd.* 43: 382, 1935.
18. Langenstrass, K. H., and Nemmann, M.: Réticulo-endothelial Sarcoma of Spleen, Report of Case, *Arch. Path.* 20: 752, 1935.
19. Alessandri, R.: Surgery of Spleen, Unusual Case (Sarcoma), *J. Mt. Sinai Hosp.* 4: 489, 1938.
20. Biller, S. B.: Primary Endothelioma of Spleen, *Arch. Path.* 25: 534, 1938.
21. Jones, A. P.: Unusual Spleen Cases, *Ann. Surg.* 109: 960, 1939.
22. Jackson, R. H.: *Ann. Surg.* 109: 964, 1939; discussion of Jones's paper; also personal communication, July 13, 1939.

A COMPARISON OF THE HINTON, KAHN, KLINE, AND MAZZINI TESTS FOR SYPHILIS*

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IT HAS been the practice of this laboratory to perform the Hinton, Kahn standard, and Kline diagnostic tests on all blood specimens submitted for serologic examinations. The specificity and sensitivity of these tests have been established by many comparative studies and by several nationwide evaluation studies.

Mazzini¹ has recently described a slide test for syphilis which is claimed to be high in sensitivity and specificity, and which has many desirable features from the standpoint of performance. Giordano² and his co-workers have confirmed Mazzini's findings.

Since it appeared that the Mazzini test might be a valuable addition to our present procedure, this investigation was made to determine what agreement we might secure with the four tests.

PROCEDURE

When specimens were received, the sera were separated and inactivated in an electrically controlled water bath at 56° C. for thirty minutes. The Hinton, Kahn standard, Kline diagnostic, and Mazzini tests were employed in the examination of each specimen. The Hinton and Kahn antigens were prepared in this laboratory and were checked against "standard" antigens for sensitivity and specificity; the Kline antigen was purchased from LaMotte and the Mazzini antigen was furnished by Mr. L. Y. Mazzini. In the performance of the tests, the technique of the originators was strictly followed. The results are given in Tables I and II.

DISCUSSION

Tables I and II show a complete agreement of all four tests in 2,818 sera (93.93 per cent), and a relative agreement in 2,858 (95.26 per cent). In an earlier study³ we reported that the Eagle, Ide, Kahn, Kline, and Laughlen tests agreed in 98.5 to 99.5 per cent of the sera examined. Lack of agreement was noted chiefly in sera which gave doubtful or weakly positive reactions.

In this study agreement was slightly lower than in our comparison of 1937. Lack of agreement usually occurred in sera which gave doubtful or weakly positive reactions. In several cases, however, one or more of the tests gave strongly positive reactions, while the others gave negative or doubtful ones.

*From the Arizona State Laboratory, Tucson.
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TABLE I
COMPLETE AGREEMENT OF HINTON, KAHN, KLINE, AND MAZZINI TESTS

	NUMBER OF SPECIMENS	PER CENT
All negative	2,184	72.80
All doubtful	17	0.56
All weakly positive	309	10.30
All strongly positive	308	10.27
Total	2,818	93.93
Results not agreeing	182	6.07
Complete total	3,000	100.00

TABLE II
RELATIVE AGREEMENT OF TESTS

	NUMBER OF SPECIMENS	PER CENT
All negative	2,184	72.80
All showing partial (\pm) or complete (+) reactions	674	22.46
Total	2,858	95.26
Results not agreeing	142	4.74
Complete total	3,000	100.00

We feel that this lack of agreement is due to two factors: (1) A larger percentage of specimens from treated, latent, and congenital cases. In 1937 specimens were chiefly from clinically diagnosed syphilitics or from presumably negative groups. With the expansion of the Venereal Disease Control Program, an increasing number of specimens come from known treated or suspected congenital cases. Mazzini¹ and Maltaner⁵ have shown that in such groups the results of various tests may vary widely. A comparison with the 1937 study supports this idea.

	1937 %	1939 %
All tests negative	82.3	72.80
All tests doubtful	1.7	0.56
All tests positive	14.3	20.57
Results not agreeing	1.7	6.07

(2) With the expansion of the Venereal Disease Control Program, more specimens are submitted for the laboratory diagnosis of syphilis and a large percentage come from cases of chronic, undiagnosed illnesses. This results in an increase of nonspecific, or "false positive," laboratory tests.

Much has been written about the specificity of the various laboratory tests for syphilis. Evaluation studies have shown that the more common tests will give between 99 and 100 per cent negative reactions among presumably non-syphilitics, and that they will detect approximately 80 to 90 per cent of known syphilitics (all types, latent, treated, etc.). In secondary syphilis the tests will detect almost 100 per cent of the cases. In such groups, our results have been well within the limits of sensitivity and specificity recommended by the United States Public Health Service.

TABLE III

DISTRIBUTION OF REACTIONS IN 182 SPECIMENS WHERE RESULTS WERE NOT IN COMPLETE AGREEMENT

(\pm and 1+ reactions called doubtful; 2+ and stronger called positive; Hinton test reported as --, \pm , or +; other tests as --, \pm , 1+, 2+, 3+, 4+)

HINTON			KAHN			KLINE			MAZZINI		
--	\pm	+	--	\pm	+	--	\pm	+	--	\pm	+
74	43	65	66	89	27	72	77	33	44	68	70
40.7	23.6	35.7	36.2	48.9	14.9	38.6	32.3	18.1	24.3	37.3	38.4

TABLE IV

DISTRIBUTION OF REACTIONS IN 3,000 SPECIMENS

	HINTON	KAHN	KLINE	MAZZINI
Negative	2,258	2,250	2,256	2,228
Doubtful or weakly positive	369	415	403	394
Positive	373	335	341	378
Total	3,000	3,000	3,000	3,000

Many diseases other than syphilis have been credited with the responsibility of nonspecific serologic tests for syphilis. Among these are yaws, tuberculosis, malaria, leprosy, infectious mononucleosis and numerous other diseases (particularly high febrile conditions). Recently, Krag and Lonberg⁴ have stated "affections of the respiratory passages are the most frequent cause of nonspecific reactions."

The question of nonspecific reactions is complicated by the fact that in some cases it is not possible to eliminate syphilis definitely, and that the sera of nonsyphilitics may contain "reagin" at times. Judging from our experience, nonspecific reactions occur, and chiefly among presumably nonsyphilitic individuals who are suffering from chronic, undiagnosed illnesses. At first, these were dismissed as laboratory errors, but after an extensive series of checks with other laboratories, we have come to regard them as nonspecific.

Tables V and VI give in detail some of the reactions given by the tests in known syphilitics and in presumably nonsyphilitics. These cases, where there was a lack of agreement, are the only ones included, except for the presumably "nonspecific" reactions. The diagnosis and comments are those furnished by the physicians who submitted the specimens.

In addition to the results given in the preceding tables, the Kline and Mazzini tests were employed as "screening tests" in a series of 1,042 specimens from a group of college students. The results are indicated in Table VII. Complete agreement in this series was 99.51 per cent; relative agreement was 99.80 per cent.

The Kahn and Hinton tests were applied to the ten specimens which gave some reaction to the Kline or Mazzini tests. The results are given in Table VIII.

These specimens were all rechecked within a period of from seven to fourteen days. The following sera gave negative Hinton, Kahn, Kline, and Mazzini reactions upon the second test: 4, 5, 6, 7, 9, and 10. The others gave the reactions shown in Table IX.

TABLE V

REACTIONS OF THE HINTON, KAHN, KLINE, AND MAZZINI TESTS IN SERA OF KNOWN SYPHILITICS

LAB. NO.	HINTON	KAHN	KLINE	MAZZINI	REMARKS
26346	+	2+	3+	1+	Congenital
31873	--	4+	--	1+	Treated
31936	+	2+	3+	1+	Treated
32087	+	1+	--	4+	Congenital
32156	--	±	1+	--	Treated
32304	--	--	2+	--	Congenital
32331	+	1+	--	3+	Treated
32422	+	2+	--	4+	Congenital
32423	+	--	4+	4+	Congenital
32966	--	--	1+	2+	Treated
33222	--	--	1+	1+	Treated
33303	--	--	1+	--	Congenital
39972	+	±	2+	4+	Treated
40210	--	±	2+	--	Congenital
40320	--	--	--	±	Treated
40623	--	--	--	4+	Treated (prostitute)
41051	+	±	3+	4+	Treated
41224	+	±	2+	4+	Treated
41437	--	--	2+	4+	Treated
41680	+	2+	--	3+	Treated
41707	--	--	±	3+	Old case, untreated
41785	+	--	--	2+	Treated
42531	±	--	--	2+	Treated
42648	+	--	2+	3+	Treated
43371	--	--	--	1+	Treated
43865	--	--	--	3+	Treated
43936	±	--	--	3+	Congenital
43219	+	±	--	3+	Treated
43922	+	--	--	3+	Old case, untreated
44231	+	±	2+	3+	Treated
44942	+	--	--	2+	Treated
44664	+	--	±	3+	Primary, untreated
45157	--	--	--	2+	Primary, untreated
45344	+	1+	1+	3+	Treated
47949	--	--	2+	3+	Treated
49699	+	--	2+	--	Treated
49744	+	3+	--	--	Treated
50902	--	2+	--	--	Treated
53526	+	3+	--	3+	Congenital
54144	±	2+	--	3+	Congenital
54162	--	2+	2+	2+	Primary, untreated
54678	+	±	2+	1+	Old case, untreated
56113	--	2+	--	2+	Congenital
59917	--	2+	--	--	Treated
69209	±	--	4+	2+	Old case, untreated
69973	±	1+	2+	1+	Treated
70588	+	2+	2+	--	Treated
70776	--	--	1+	--	Congenital
74646	+	1+	2+	1+	Congenital
74671	--	1+	2+	1+	Congenital

At the time this article was written, further investigations had not been completed. With the exception of specimen No. 8, there is good reason to believe that the reactions by sera Nos. 1, 2, and 3 are nonspecific.

Tables III and IV show that the Kline, Kahn, and Hinton tests gave approximately the same number of negative reactions, and that the Mazzini test gave fewer negative and more positive reactions. This is to be expected, however, since the sensitivity of the Mazzini test is of the same order as the Kline

TABLE VI

NONSPECIFIC REACTIONS IN PRESUMABLY NONSYPHILITIC INDIVIDUALS
(NO HISTORY OR CLINICAL SYMPTOMS OF SYPHILIS)

LAB. NO.	HINTON	KAHN	KLINE	MAZZINI	REMARKS
26210	±	2+	2+	1+	Normal*
27579	--	--	--	2+	Normal
28835	±	--	2+	±	Influenza†
28844	+	2+	2+	--	Endocarditis
39402	--	--	--	4+	Sinusitis
40237	±	±	--	±	Sinusitis†
41069	±	--	--	2+	Normal
41555	±	3+	±	±	Undulant fever†
43008	±	±	2+	3+	Jaundice†
43773	±	±	--	2+	Normal
46520	--	+	+	2+	Chronic ear abscess
58355	+	3+	3+	3+	Tularemia†
59323	+	1+	3+	3+	Normal
60344	+	--	3+	3+	Normal
60353	+	2+	2+	2+	Normal
60362	+	1+	1+	3+	Normal†
60371	±	1+	1+	3+	Normal†
60380	+	2+	3+	4+	Normal†
60389	+	1+	4+	4+	Normal†
69173	+	--	1+	3+	Recheck (No. 60344)
69182	--	1+	--	2+	Recheck (No. 60353)
69191	--	1+	2+	3+	Recheck (No. 59323)
71359	+	2+	2+	2+	Normal†
71368	+	2+	2+	--	Normal†
71858	--	1+	±	±	Psoriasis†

*The term normal designates specimens from healthy individuals. These were submitted in connection with physical examinations of students, food handlers, civil service applicants, etc. At the time of examination there was no clinical evidence of illness. Investigations made subsequently revealed no evidence of syphilis.

†Indicates that another specimen, submitted at a later date, gave negative reactions to all tests.

TABLE VII

KLINE AND MAZZINI TESTS ON SERA FROM COLLEGE STUDENTS

	NUMBER OF SPECIMENS	PER CENT
Kline and Mazzini both negative	1,028	98.65
Kline and Mazzini both positive (complete agreement)	9	0.86
Kline and Mazzini both positive (partial agreement)	3	0.29
Kline and Mazzini not in agreement	2	0.20
Total	1,042	100.00

TABLE VIII

HINTON, KAHN, KLINE, AND MAZZINI REACTIONS ON SERA OF COLLEGE STUDENTS

SERUM	HINTON	KAHN	KLINE	MAZZINI
1				
2	+	1+	3+	3+
3	+	--	3+	3+
4	+	2+	2+	2+
5	+	1+	1+	3+
6	+	1+	1+	4+
7	+	2+	3+	4+
8	+	1+	4+	4+
9	+	2+	4+	4+
10	+	2+	2+	2+
	+	2+	2+	--

exclusion test. Table IV shows a good relative agreement between the Kline, Kahn, and Hinton tests.

Table V confirms the results of Mazzini¹ and Giordano² as to the sensitivity of the Mazzini test, and also shows, as have Mazzini¹ and Maltaner,⁵ that many persons known to have syphilis may give positive reactions with one or more tests and negative reactions with others. It is interesting to note that in such cases a less sensitive test may often give a positive reaction, while a more sensitive test may be negative.

Table V also shows the advantage of the use of a system of multiple tests, as suggested by Pierce.⁶ The use of such a system has been criticized by several authors on the grounds that conflicting results are of little value to the physician. After all, he is the final judge of the laboratory report and must interpret such results in the light of clinical evidence. Although Table VI shows few cases which are presumably "false positive," or nonspecific reactions, there are many instances where clinically diagnosed cases of syphilis give similar conflicting results with the various tests. From this experience, we feel that the use of a multiple test system is warranted, and that conflicting results from several tests should not necessarily be regarded as a limitation of any particular laboratory test.

It is our opinion that as the Venereal Disease Program continues to expand, there will be an increase in the type of results given in Table V, and that an appreciation of this fact will lead to a better understanding between the laboratory and the clinician.

TABLE IX

SERUM	HINTON	KAHN	KLINE	MAZZINI	WASSERMANN
1	--	1+	2+	3+	--
2	±	--	1+	3+	--
3	--	1+	--	2+	--
8	+	4+	4+	4+	4+

SUMMARY

These results confirm the findings of Mazzini and Giordano as to the specificity and sensitivity of the Mazzini test.

Since the Mazzini test is more sensitive than the Kline and Kahn diagnostic tests and the Hinton test, it gave a lower incidence of negative reactions and a higher incidence of positive findings.

The Kline, Kahn, Hinton and Mazzini tests gave a relative agreement of approximately 95.26 per cent, and an absolute agreement of 93.93 per cent. In an additional series of 1,042 specimens from college students, the Kline and Mazzini tests gave a relative agreement of 99.8 per cent and a complete agreement of 99.51 per cent.

The occurrence of nonspecific ("false positive") reactions is mentioned.

The occurrence of conflicting results of various tests in clinically diagnosed cases of syphilis is discussed.

The results, in our opinion, justify the use of a multiple test system.

In our opinion the Mazzini test is an excellent test for the laboratory diagnosis of syphilis.

We gratefully acknowledge the assistance of Mr. L. Y. Mazzini, who supplied the antigen for the Mazzini test, and the physicians who assisted us by giving case histories.

REFERENCES

1. Mazzini, L. Y.: A Reliable, Sensitive, Simple and Rapid Slide Flocculation Test for Syphilis, *Am. J. Clin. Path.* 9: 163, 1939.
2. Giordano, A. S., Wilhelm, A. M., and Carlson, B. G.: Comparative Study of the Mazzini Flocculation and the Kline Exclusion Test, *Am. J. Clin. Path., Tech. Suppl.* 3: 80, 1939.
3. Breazeale, E. L., Greene, R. A., and Harding, H. B.: A Comparison of the Eagle, Ide, Kahn, Kline and Laughlen Tests for Syphilis, *Southwestern Med.* 22: 311, 1938.
4. Krag, P., and Lonberg, A.: The Occurrence of Strong Unspecific Wassermann-Kahn Reaction, *Acta dermat.-venereol.* 19: 612, 1938.
5. Maltaner, E.: Serologic Tests in the Diagnosis of Syphilis, *Am. J. Pub. Health* 29: 104, 1939.
6. Pierce, L. F., Patterson, H. A., Stevenson, R. A., and Torbert, H. C.: A Multiple Test Method for Routine Use in the Serodiagnosis of Syphilis, *Am. J. Syph., Gonorr. & Ven. Dis.* 22: 59, 1938.

BACTERIOLOGIC DIAGNOSIS IN GONORRHEA OF THE MALE*

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A BACTERIOLOGIC study of 221 patients with genitourinary infections is presented, and a comparison is made between the efficiency of the smear method and the cultural method as a means of diagnosis. These patients were repeatedly examined over a period of eight months. A total of 819 bacteriologic examinations, each examination consisting of smear and culture, were made on the 221 patients, of whom 160 were definitely proved to have gonorrhea by bacteriologic examination as well as by clinical evidence. Sixty-one patients were assumed to have some genitourinary infection other than gonorrhea and, while the actual cause of the infection was not established, at no time were gonococci found either by smear or culture.

METHODS

Smears: Material for smears consisted of urethral discharge, sediment of urine, and prostatic secretion, obtained after massage. The material was collected on a swab or loop and distributed with rolling or streaking motions over the glass slide.

Staining procedure: Hucker's staining method, which we modified by employing mercuric chloride as a fixative and acetone as a decolorizing agent, was used. It was found that mercuric chloride in 1 per cent solution applied for one minute to the smears would preserve the details of the cells especially well.

Cultural methods: In attempting to grow gonococcus the work of McLeod and co-workers,¹ Leahy and Carpenter,² and Carpenter,³ was taken into account. The specimens for culture, which were the same as those for the smears, were collected on a sterile swab. The swab was then placed into 1.5 c.c. of broth, and the material on the swab was suspended in the broth. For technical reasons it

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was not possible to plate out the material at once, but the broth suspensions were exposed to room temperature for one and one-half to two hours before being cultured. Only in a very few cases was the glans penis cleaned, since in most cases there was not sufficient time to do it. By proper manipulations a large number of contaminations could be avoided, so that the contaminants growing on the plates would not interfere with the growth of the gonococcus. Cultures were taken only after the patients had stopped treatment for four to five days.

A 5 per cent rabbit blood chocolate agar was used, with hormone broth as a base. The blood was added to the agar, kept at a temperature of between 70° and 85° C. The final agar concentration in the medium was 1.7 per cent. The chocolate agar plates were kept in the icebox. They were not used when older than three days, since it was found that only a high amount of moisture would guarantee satisfactory growth.

Some of the broth suspension was placed on the medium by means of the swab and was then streaked out with the loop. Incubation was effected at 36° C. for about forty hours under 10 per cent carbon dioxide tension.

After forty hours' incubation the plates were inspected, and colorless, opaque colonies with irregular edges were touched with a drop of a 1 per cent watery solution of para-amino-dimethyl-aniline-monohydrochloride (Eastman Kodak), prepared fresh as soon as the solution showed some precipitate. This solution was kept in the refrigerator. If no suspicious colonies were found by mere inspection, the entire plate was flooded with the solution for a moment, and then the excess solution was poured off. Gonococcus colonies gave a typical oxidase reaction; the colonies turned pink in about ten seconds, and in the course of some minutes gradually became black. Gram-stained preparations of colonies giving a typical oxidase reaction were invariably found to consist of gram-negative diplococci. The oxidase reaction does not interfere with the characteristic staining properties of the gonococcus.

Besides gonococcus, other organisms will give the oxidase reaction. All or most of the members of the *Neisseria* group will give this reaction. With the exception of the meningococcus, whose colony cannot be very well differentiated from the gonococcus colony on a chocolate agar medium, and which also gives an oxidase reaction very similar to that of the gonococcus, the other members of the *Neisseria* group can be easily recognized from the gonococcus by their growth characteristics; they also give an oxidase reaction that is somewhat different from that of the gonococcus.

While diphtheroids do not generally give the oxidase reaction, occasionally colonies of gram-positive rods, which we assumed to be diphtheroids, were found to change their color when the oxidase stain was applied to them. However, besides being gram-positive, they could be differentiated easily from the gonococcus, giving an absolutely atypical reaction, and turning to a chalky, grayish black after fifteen to twenty minutes. When these colonies were pushed aside with the loop, the underlying medium would generally not have taken up any of the dark color, whereas the medium under gonococcus colonies, after they gave the oxidase reaction, would also be stained black. Furthermore, if a gonococcus

colony is pushed aside, a distinctly positive oxidase reaction may be obtained by applying the oxidase stain to that place of the medium where the gonococcus colony had been before. This we found did not occur with diplotheroids which gave the atypical reaction just mentioned.

In 41 cases where colonies on the basis of their morphologic character and on their property of giving a typical oxidase reaction were thought to be gonococci, the suspected colonies were isolated in pure culture and fermentation tests on an ascitic fluid agar sugar medium, as well as sodium hydroxide solubility tests, were done. In every case the tests gave the reactions characteristic of gonococcus. Besides, as a further control, all the 41 cultures were transferred to a 7 per cent horse blood agar plate and incubated at 23° to 24° C. for forty-eight hours, but without the adjustment of carbon dioxide tension. With the exception of two strains the organisms failed to grow.

On the basis of these preliminary findings in a subsequent routine procedure, all colonies, which resembled gonococcus colonies by inspection, by typical oxidase reaction, and showed gram-negative diplococci were considered to be gonococci without using fermentation tests as a means of further identification, provided these organisms were derived from the genitourinary tract of a male with a typical history indicating gonorrheal infection.

We are aware of the possibility that this method might fail to differentiate meningococci from gonococci; but in view of the rarity of meningococcal infection of the male genitourinary tract, we thought it justifiable to give out a report as positive, without going through the very time-consuming procedure of first growing the organisms in pure culture and then running fermentation tests, providing our previously stated criteria of gonococcus were satisfied.

METHOD OF REPORTING

Cultures: A culture was reported as "+" when gonococcus colonies were found, and as "0" when no such colonies were found.

Smears: A smear was reported as "+" when intracellular gram-negative diplococci resembling gonococci were found; as "±" when gram-negative diplococci resembling gonococci were found extracellularly; as "0" when such organisms were found neither intracellularly nor extracellularly.

RESULTS

A total of 819 examinations, employing both culture and smear methods simultaneously, were carried out on 221 patients.

Stain and culture agreed in 722 instances, or in 88.16 per cent.

Stain	+	} 137 = 16.73%	Stain	0	} 585 = 71.43%
Culture	+		Culture	0	

Stain and culture disagreed in 97 instances, or in 11.84 per cent.

Stain	0	} 30 = 3.66%	Stain	+	} 5 = 0.61%
Culture	+		Culture	0	
Stain	±	} 13 = 1.59%	Stain	±	} 49 = 5.98%
Culture	+		Culture	0	

The presence of gonococcus was revealed by either smear or culture method, or both methods, in a total of 185 instances of the 819 examinations. The relative efficiency of the smear or culture method for detection of the presence of gonococcus may be calculated from the following figures, taking the number 185 as 100 per cent deficiency:

Stain	+	} 137 = 74.05%	Stain	0	} 30 = 16.22%	} Difference 13.52%
Culture	+		Culture	+		
Stain	±	} 13 = 7.03%	Stain	+	} 5 = 2.70%	
Culture	+		Culture	0		

Thus, we see that there is a decided superiority of 13.52 per cent of the cultural method over the smear method. In other words, in 13.52 per cent the culture method detected gonococci, when the smear method failed to do so. In addition, in 7.03 per cent the culture method definitely revealed gonococci when the smear method was doubtful.

Three of the 5 stain + culture 0 findings occurred during the first two months of our investigation, when we were not as familiar with the growth conditions of gonococcus as we were later; and it is believed that in these instances the failure of the gonococcus to grow might have been due, perhaps, to too long storage of the medium with a subsequent loss of moisture.

As we have already stated, the report "±" referring to the smear signified "gram-negative diplococci, resembling gonococci, situated extracellularly." Thus, in reporting a smear as "±," it was necessary in the first place to decide whether the extracellular organisms were gram-negative, then whether they were diplococci, and finally whether they resembled the gonococci. While it is often an easy matter to arrive at the report "±," there is a considerable number of smears where the decision is difficult, and the personal equation enters as in no other phase of the work. In many smears where the culture exhibited staphylococci and diphtheroids only, the smear showed besides gram-positive cocci, cocci that were not definitely gram-positive or gram-negative, associated at times in diplococcus formation. The decision whether these organisms are also staphylococci, which just did not stain as well as the others, or whether they should be regarded as being possibly gonococci, is entirely a matter of personal judgment, and even the same observer looking at a given smear today may come to a different decision tomorrow.

Taking the total of 819 smears and cultures as 100 per cent, we see that in 62 instances (7.57 per cent) the smear was reported as "±." In 13 of these instances (1.59 per cent) the diagnosis was settled by the simultaneous positive culture. In 49 instances (5.98 per cent) the culture proved to be negative. These 49 doubtful "±" smears occurred on 43 patients, 11 of whom were regarded as being affected with some genitourinary infection other than gonorrhea, and on whom at no time a definite positive finding had been obtained. The other 32 patients had clinical gonorrhea confirmed on admission by smear and culture, but who at a later date exhibited a "±" smear along with a negative culture.

It was at first difficult for us to correlate the " \pm " findings of the smears with the simultaneous negative findings of the cultures, since it may have been possible that the suspicious organisms found in the smear were gonococci, which just would not grow under the conditions to which they were subjected by our methods. However, when more data were available, it was felt that in judging whether the organisms responsible for the " \pm " report of a smear were gonococci or not, the deciding factor should be the culture, in the sense that if no gonococci were found on the culture, the suspected organisms in the smear should not be assumed to be gonococci. This assumption seemed to be justified by the following reasoning: It was very unlikely that in the case of a doubtful " \pm " smear and a negative culture we would deal with a strain of gonococcus that would not grow under our methods, since a positive culture was obtained in every case where these patients showed a definitely positive smear on admission.

We further undertook a follow-up study of the patients who exhibited a stain \pm culture 0 report, with the idea that if the suspicious organisms in the " \pm " smear were gonococci, the symptoms of the patients might again exacerbate and culture and stain become positive. The findings of this study are as follows:

In the large majority of patients there were several reports of negative smears and cultures preceding the " \pm " smear. Five patients were found unsatisfactory for any analysis, either because they did not return or because they allowed themselves to be exposed during the time of observation. Twenty-six patients, of whom 12 have been definitely dismissed as cured, showed only stain 0 culture 0 findings after the stain \pm culture 0 report. Fourteen of the 26 patients have not been dismissed at this time, and show repeatedly a stain 0 culture 0 report after the stain \pm culture 0 report. Most of these have been put through provocative measures as massage, introduction of sound, permission of alcohol and coitus, without either culture or stain becoming positive. Three of the 14 patients not yet dismissed had, we believe, reinfections. For a period of two and one-half to three months after the stain \pm culture 0 report, during which these patients were examined repeatedly, no positive or " \pm " findings occurred. These patients were finally permitted to stay away for twenty-eight days; they returned with an acute discharge. One of the patients admitted exposure without prophylaxis.

Only in one of the 32 patients was there a stain 0 culture + finding after the stain \pm culture 0 report.

We admit that this method of analyzing the significance of a " \pm " finding on the smear by follow-up studies is very much open to criticism. However, we thought that some additional information might thus be gained.

Reviewing this analysis, we see that in only 5 instances of 819 bacteriologic examinations, an incidence of 0.61 per cent, where the stain was positive and the simultaneous culture was negative, did the stain detect something important that the culture did not reveal.

CONCLUSIONS

1. A total of 819 bacteriologic examinations, employing simultaneously culture and smear methods as a means of diagnosis, were carried out on 221 patients.

2. The culture and smear methods agreed in 88.16 per cent and disagreed in 11.84 per cent of the cases, taking the 819 examinations as 100 per cent.

3. In 185 instances the presence of gonococci was revealed by either smear or culture, or both methods. Taking the number 185 as 100 per cent efficiency in detecting gonococci, we find that the culture was able to detect gonococci in 13.52 per cent, when the smear definitely failed to do so. In addition, in 7.03 per cent, the culture definitely revealed gonococci, when the smear was doubtful.

REFERENCES

1. McLeod, J. W., Coates, J. C., Happold, F. C., Priestley, D. P., and Wheatley, B.: Cultivation of the *Gonococcus* as a Method in the Diagnosis of Gonorrhea With Special Reference to the Oxydase Reaction and to the Value of Air Reinforced in Its Carbon Dioxide Content, *J. Path. & Bact.* 39: 221, 1934.
2. a. Leahy, A. D., and Carpenter, C. M.: The Diagnosis of Gonococcal Infections by the Cultural Method, *Am. J. Syph., Gonorr. & Ven. Dis.* 20: 347, 1936.
b. Carpenter, C. M., and Leahy, A. D.: A Comparison of the Results of the Smear and Cultural Methods for the Diagnosis of Gonococcal Infection in Adult Females, *Tr. Am. Neisserian M. Soc.* 3: 5, 1937.
3. Carpenter, C. M.: The Diagnosis of Gonococcal Infection in the Male, An Evaluation of Laboratory Methods, *J. A. M. A.* 109: 1428, 1937.

EXPERIMENTAL THROMBOCYTOPENIC PURPURA IN THE GUINEA PIG*

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SINCE Werlhof first gave an accurate clinical description of essential thrombocytopenic purpura, it has been known that a marked reduction of the circulating blood platelets is a characteristic feature of the condition. However, many students of this disease believe that other factors must be in operation to produce the clinical picture, and it has been assumed that some change in the capillaries must be the ultimate cause of the leakage of blood through them.

This study was planned as an attempt to estimate the relative roles played by the blood platelets and the capillaries. Our approach to the problem was as follows: (1) by producing thrombocytopenia, and (2) by producing increased capillary fragility.

Experimental Production of Thrombocytopenia.—Numerous investigators have studied the effects of various substances upon blood platelets.²⁻²¹

Duke² was apparently the first in this country to attempt to produce thrombocytopenic purpura, and for this purpose he used benzol. Ledingham³

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in 1914 reported the results of an experiment in which thrombocytopenic purpura was produced in guinea pigs following injections of an antiplatelet serum which was capable of selectively destroying circulating platelets. Ledingham and Bedson,⁴ and Bedson,^{15, 16, 20} after studying the properties of this serum, concluded that it contained a specific antibody capable of lysing and agglutinating blood platelets *in vivo*, without affecting the other formed elements of the blood. Tocantins^{7, 8} studied the thrombocytopenic purpura produced in dogs with an antiplatelet serum, and more recently Troland and Lee²¹ produced thrombocytopenic purpura in rabbits with an extract obtained from the spleen of patients with thrombocytopenic purpura.

Experimental Production of Increased Capillary Fragility.—Our problem was to find a method for producing capillary damage that could be maintained over long periods, that would in itself not cause purpura, and that could be controlled to some extent. We employed vitamin C deficiency as the method that best fulfilled these conditions. Our attempt was to produce vitamin C depletion without reaching the point of definite clinical scurvy. Vitamin C has been regarded by many as an important factor in the hemorrhagic diseases, from both the etiologic and therapeutic standpoints.²²⁻¹² In recent years capillary fragility has been employed extensively as an indirect measure of the degree of vitamin C storage.²³⁻¹¹

METHODS

An antiplatelet serum was prepared in the following manner: One hundred to 130 c.c. of blood were obtained from guinea pigs by cardiac puncture. The blood was collected in an equal volume of 3.8 per cent sodium citrate in normal salt solution. The platelets were extracted from the solution by fractional centrifugalization, and after being suspended in 10 c.c. physiologic salt solution, were injected intravenously into a rabbit. This procedure was carried out at intervals of five to seven days, until the rabbit had received from three to six injections. At the end of this time the blood was withdrawn from the rabbit by cardiac puncture, stored in the refrigerator overnight, and the serum separated out and collected in sterile bottles. Because it was found that the antiplatelet serum lost potency in a relatively short time, all subsequent sera were lyophilized according to the technique of Flosdorff and Mudd.⁴²

Hemoglobin determinations were made with standardized Sahli instruments in which 100 per cent equal 13.8 Gm. of hemoglobin per 100 c.c. of blood. Erythrocyte and leucocyte counts were made with standard pipettes and a Neubauer counting chamber, all instruments being standardized by the United States Bureau of Standards. Platelets were counted directly in the counting chamber after diluting 1:100 in the standard red blood cell pipettes with Reese and Ecker fluid.¹ A period of ten minutes was allowed to elapse from the time the fluid was placed in the counting chamber until the count was begun.

Capillary resistance was measured by the Dalldorf technique,³⁷ employing the da Silva-Mello instrument. We used the skin over the lower portion of the abdomen, which had been carefully shaved at least twenty-four hours before the readings were taken.

Twelve young guinea pigs, weighing between 200 and 300 Gm., were chosen. Six were segregated and placed upon a diet consisting of baked skimmed milk, 30.0 per cent; butter fat, 10.0 per cent; rolled oats and bran, 56.0 per cent; sodium chloride, 0.5 per cent; Osborne and Mendel salt mixture, 1.5 per cent; dried yeast, 1.0 per cent; and cod-liver oil, 1.0 per cent. This group was designated as the scurvy (S) group.

Six animals were placed upon a diet consisting of barley, alfalfa, salt, and greens (lettuce and cabbage) supplemented with 2 c.c. of orange juice daily. This was designated as the control (C) group.

TABLE I

EFFECT OF DIET ON WEIGHT, BLOOD, AND CAPILLARY FRAGILITY OF GUINEA PIGS

TWO GROUPS OF SIX GUINEA PIGS EACH	ANIMALS ON ADEQUATE VITAMIN C DIET			ANIMALS ON VITAMIN C DEFICIENT DIET		
	NUMBER OF DETERMI- NATIONS	STANDARD DEVIATION ± P.E.	MEAN ± P.E.	NUMBER OF DETERMI- NATIONS	STANDARD DEVIATION ± P.E.	MEAN ± P.E.
Weight in grams	40	114 ± 8.65	371 ± 77	26	57 ± 5.3	316 ± 38.3
Hemoglobin percentage	34	4.36 ± 0.38	74 ± 0.49	36	5.93 ± 0.47	73 ± 0.68
Erythrocytes	32	310,000 ± 26,400	5,640,000 ± 35,000	37	500,000 ± 39,400	5,370,000 ± 56,000
Leucocytes	33	2130 ± 178	9100 ± 240	36	4650 ± 372	9600 ± 530
Platelets	47	101,500 ± 7,100	473,000 ± 9,735	47	178,600 ± 12,500	460,000 ± 17,683
Capillary fragility	16	0 - 5 at 15 cm.		16	3 - 35+ at 15 cm.	

EXPERIMENTAL

Effect of Diet on the Weight, Blood Picture, and Capillary Fragility of Guinea Pigs.—The results are shown in Table I. There was a definite influence of vitamin C deficiency on growth. These animals were at the age when rapid growth is expected, and, although the vitamin C deficiency animals showed some increase in weight, it was neither as uniform nor as great as that shown by the animals receiving vitamin C.

The values for hemoglobin, erythrocytes, leucocytes, and blood platelets were statistically similar in the two groups, whereas the capillary fragility showed considerable difference. The vitamin C deficiency animals showed a distinctly greater capillary fragility when measured by the Dalldorf method. The effect of the deficiency was more marked on the capillaries than on the formed elements of the blood.

Effect of Antiplatelet Serum on Guinea Pigs With Vitamin C Deficiency and With High Vitamin C Diets.—Six guinea pigs, three with vitamin C deficiency and high capillary fragility and three on diets high in vitamin C and with low capillary fragility, were chosen. The animals were given antiplatelet serum intraperitoneally. Four received 0.2 c.c. and two of the control group received 0.3 c.c. The results (Table II and Fig. 1) were strikingly similar. Before the injection of antiplatelet serum, the average platelet count was 600,000 per cubic millimeter in the vitamin C deficient animals and 710,000 per cubic millimeter in the control animals. Twenty-four hours after the injection the platelet count had fallen to 70,000 and 40,000 per cubic millimeter, respectively. This was

associated with the appearance of purpura, increased bleeding time, and poor clot retraction. The clot was so soft and friable that twenty-four hours after the previous skin puncture, the clot could be wiped away and persistent oozing would follow. Forty-eight hours after the injection, the platelets began to increase again, reaching by the fifth day 380,000 per cubic millimeter in the vitamin C deficient animals and 400,000 per cubic millimeter in the controls. A second injection of the serum (0.2 c.c.) again produced a rapid drop in platelets; this was followed by the appearance of purpura. The platelet drop was very marked within six-hours after the injection, but was maximal at twenty-four hours.

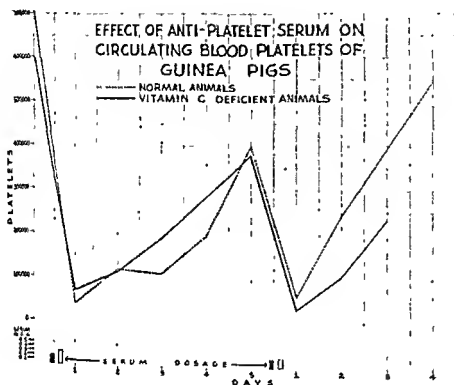


Fig. 1.

Two of the animals (control) died, one forty-eight hours and one fifty-two hours after the injection. Necropsy showed bleeding into the internal organs and serous membranes. Death was apparently due to exsanguination. In all surviving animals the purpuric lesions disappeared in from three to five days.

Effect of Normal Rabbit Serum on Guinea Pigs Deficient in Vitamin C.—In order to determine whether normal rabbit serum had any effect upon blood platelets, injections ranging in dosage from 0.2 to 1.0 c.c. were given to five animals with vitamin C deficiency. The data are shown in Table III. There was no significant variation in the platelet levels and no evidence of a bleeding tendency. This definitely indicates that (1) the platelet-destroying property of the antiserum is not inherent in the rabbit serum; (2) normal rabbit serum will not alter the capillary permeability sufficiently to cause bleeding.

Effect of Increasing Doses of Antiplatelet and Normal Rabbit Serum on Vitamin C Deficient Guinea Pigs.—Two vitamin C deficient animals were given injections of antiplatelet serum in increasing doses, ranging from 0.05 c.c. to 0.3 c.c. One animal was given normal rabbit serum in the same manner. (Results are shown in Tables IV and V and Fig. 2.) The smallest dose (0.05 c.c.)

TABLE II
EFFECT OF ANTIPLATELET SERUM ON VITAMIN C DEFICIENT GUINEA PIGS

ANIMAL	AMOUNT IN C.C.	INTERVAL AFTER INJECTION	PLATELETS	REMARKS
S-11	0.2	Before	530,000	Entire body covered with petechiae and purpuric spots. Bleeding time prolonged No fresh petechiae Petechiae fading Petechiae gone. Ecchymoses fading
		24 hr.	68,000	
		48 hr.	126,000	
	0.2	3 days	182,000	Entire skin covered with fresh petechiae Petechiae starting to fade Petechiae gone
		4 days	364,000	
		5 days	32,000	
S-12	0.2	24 hr.	32,000	Entire body covered with purpuric spots. One on back 3 cm. in diameter Hemorrhagic spots fading Ecchymoses fading but still visible Spots faded
		48 hr.	80,000	
		3 days	302,000	
	0.2	Before	692,000	Entire body covered with fresh spots and pin-point petechiae. Bleeding time prolonged Petechiae fading. Bleeding time not prolonged Petechiae gone. Ecchymoses still visible
		24 hr.	62,000	
		3 days	175,000	
S-13	0.2	4 days	278,000	Body covered with purpuric spots and fine petechiae. Bleeding prolonged. Animal lively No fresh petechiae Hemorrhagic spots fading Larger ecchymoses still visible Hemorrhagic spots gone
		5 days	44,000	
		24 hr.	142,000	
	0.2	48 hr.	282,000	Bleeding prolonged. Pin-point petechiae and small bright red ecchymotic spots over entire body No new spots Petechiae almost entirely faded
		3 days	594,000	
		24 hr.	86,000	

EFFECT OF ANTIPLATELET SERUM ON GUINEA PIGS ON HIGH VITAMIN C INTAKE

C-11	0.3	Before	550,000	Ear bled for one hour after puncture Blood still oozing from ear Animal dead. Organs exsanguinated. Ecchymoses into pericardium, lungs, skin, renal cortices, and wall of stomach and bowel. Stomach filled with blood clot
		6 hr.	48,000	
		24 hr.	40,000	
		48 hr.		
C-12	0.3	Before	760,000	No bleeding Skin covered with petechiae and ecchymoses No fresh purpura Petechiae fading. Ecchymoses still visible Purpura fading Ecchymoses gone
		6 hr.	96,000	
		24 hr.	50,000	
		2 days	120,000	
	0.2	3 days	110,000	Ear bled excessively from puncture Skin covered with fresh petechiae Petechiae fading Petechiae gone
		4 days	186,000	
		6 days	408,000	
		7 hr.	72,000	
C-13	0.2	24 hr.	60,000	Ear bled profusely from puncture Oozing at site of former puncture. Skin covered with petechiae and large ecchymotic areas Moribund Dead. Bled into skin, serous membranes, lungs, pancreas, liver, stomach, and colon
		48 hr.	244,000	
		50 hr.	548,000	
		Before	800,000	

TABLE III

EFFECT OF NORMAL RABBIT SERUM ON GUINEA PIGS WITH VITAMIN C DEFICIENCY

ANIMAL	AMOUNT IN C.C.	INTERVAL AFTER INJECTION	PLATELETS	REMARKS
N-1	0.2	Before	580,000	No evidence of bleeding
		6 hr.	496,000	
	0.4	24 hr.	428,000	
		6 hr.	400,000	
N-2	0.2	24 hr.	365,000	No evidence of bleeding
		6 hr.	585,000	
	0.4	6 hr.	646,000	
		24 hr.	542,000	
N-3	0.2	6 hr.	570,000	No evidence of bleeding
		24 hr.	520,000	
	0.4	Before	620,000	
		6 hr.	612,000	
N-4	0.2	24 hr.	634,000	No evidence of bleeding
		6 hr.	510,000	
	0.4	24 hr.	570,000	
		Before	408,000	
N-5	0.8	6 hr.	400,000	No evidence of bleeding
		24 hr.	480,000	
	1.0	Before	342,000	
		6 hr.	326,000	
		24 hr.	348,000	

TABLE IV

EFFECT OF INCREASED AMOUNTS OF ANTIPLATELET SERUM ON GUINEA PIGS WITH VITAMIN C DEFICIENCY

ANIMAL	AMOUNT IN C.C.	INTERVAL AFTER INJECTION	PLATELETS	REMARKS
S-16	0.05	Before	746,000	No evidence of bleeding
		0 hr.	700,000	
		24 hr.	254,000	
	0.1	6 hr.	252,000	
		24 hr.	100,000	
		Before	308,000	
	0.2	6 hr.	04,000	
		24 hr.	88,000	
S-17	0.3	6 hr.	90,000	No bleeding
		24 hr.	70,000	
		Before	400,000	
	0.05	6 hr.	370,000	
		24 hr.	304,000	
		6 hr.	222,000	
	0.1	24 hr.	56,000	
		6 hr.	40,000	
		24 hr.	34,000	

of antiplatelet serum was followed by a drop in the platelet level (from 570,000 to 280,000 per cubic millimeter), but this was not regarded as being significant. Following an injection of 0.1 c.c., the platelet level dropped to 75,000 per cubic millimeter. Larger doses produced no further significant decrease in the platelet level, but a dose of 0.2 c.c. was required to produce purpura and increased bleeding time.

Following injections of serum from a normal rabbit, there was no significant change in the level of blood platelets. There was variation, ranging from 368,000 to 650,000 per cubic millimeter (Table V and Fig. 2), but this was within the probability of normal variation, as established by the statistical constants.

Apparently the maximum effect of the antiplatelet serum cannot be exceeded by increasing the dosage, but is inherent in the potency of the serum.

TABLE V

EFFECT OF INCREASED AMOUNTS OF NORMAL RABBIT SERUM ON GUINEA PIG WITH VITAMIN C DEFICIENCY

ANIMAL	AMOUNT IN C.C.	INTERVAL AFTER INJECTION	PLATELETS	REMARKS
S-15	0.05	Before	620,000	No evidence of bleeding at any time
		6 hr.	600,000	
		24 hr.	650,000	
	0.1	6 hr.	614,000	
		24 hr.	550,000	
		Before	368,000	
	0.2	6 hr.	400,000	
		24 hr.	450,000	
	0.3	6 hr.	400,000	
		24 hr.	400,000	

TABLE VI

EFFECT OF ANTIPLATELET SERUM DILUTED WITH NORMAL RABBIT SERUM ON PLATELETS OF NORMAL GUINEA PIGS

ANIMAL	AMOUNT IN C.C.		INTERVAL AFTER INJECTION	PLATELETS	REMARKS
	ANTI-PLATELET SERUM	NORMAL RABBIT SERUM			
P-1	0.1	+	0.9	Before	No bleeding Skin covered with petechiae Bleeding time prolonged
	0.3	+	0.7	24 hr.	
				24 hr.	
P-2	0.2	+	0.8	Before	A few scattered petechiae behind the ears Numerous petechiae and several ecchymoses
	0.4	+	0.6	24 hr.	
				24 hr.	
P-3	0.5	+	0.5	Before	Skin and mucous membranes covered with petechiae and ecchymoses Bleeding time prolonged Peritoneal cavity filled with free blood The serous membranes were covered with ecchymoses
				24 hr.	
				48 hr.	
P-4	0.7	+	0.3	Before	Skin covered with petechiae and ecchymoses Bleeding time prolonged
				24 hr.	
				24,000	

Effect of Antiplatelet Serum Diluted With Normal Rabbit Serum.—We attempted to find whether dilution would result in loss of potency and whether the admixture of normal serum would result in changes in the antiplatelet properties of the antiplatelet serum. Four normal guinea pigs were given injections

of mixtures of antiplatelet serum and normal rabbit serum. The total volume of serum injected was 1 c.c., of which the antiplatelet portion ranged from 0.1 to 0.7 c.c. There was no difference between the response to the mixed serum and that which had been observed using the straight antiplatelet serum (Table VI). When the proportion of antiplatelet serum reached 0.2 c.c., there followed within twenty-four hours a marked platelet drop (from 250,000 to 60,000 per cubic millimeter) associated with hemorrhagic phenomena.

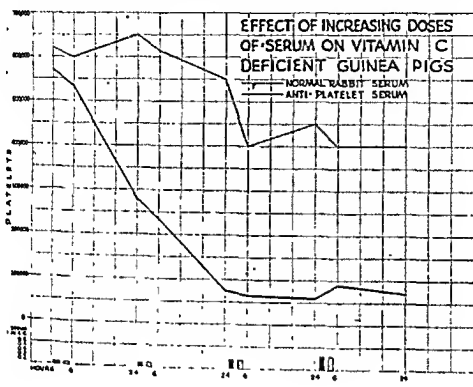


Fig. 2.

DISCUSSION

It is difficult to draw definite conclusions when dealing with experiments that present uncontrollable variables. However, we feel that certain facts appeared consistently enough to warrant attention. The most striking and consistent factor was the apparent relationship between the blood platelet level and the bleeding time. Generally, when the platelet level fell below 70,000 per cubic millimeter, a condition was produced similar to that commonly seen in human thrombocytopenic purpura, i.e., prolonged bleeding time, normal clotting time, poor clot retraction, and bleeding into the skin, mucous membranes, and internal organs. This was not always the case, as instances were observed when a low platelet count was not associated with purpuric manifestations. The rapidity of the fall in the platelet level, as well as the absolute number of circulating platelets, appeared to be important factors. This may explain why very small doses of serum, although capable of producing thrombopenia, were unable to cause bleeding.

This study was predicated upon the assumption that a capillary lesion, in addition to a platelet deficiency, is necessary for purpura to occur. The presence of a capillary lesion was assumed when an increased capillary fragility was shown by the Dalldorf technique for estimating capillary fragility. In evaluating the part played by the capillary lesion in thrombocytopenic purpura, we considered that the capillary bed in vitamin C deficiency might be very per-

meable and allow capillary hemorrhages to occur, with slight reductions in the platelet level, i.e., a level above that necessary to cause purpura in a normal animal. As has been found in these experiments, such a premise was not warranted. Unfortunately, we have no means of showing mathematical correlation between vitamin C deficiency, capillary fragility, and purpura, but so far as our experiments are concerned, there appears to be none.

This does not mean that vitamin C is not concerned with capillary fragility, but it does indicate that the type of capillary change which we assume to be present in thrombocytopenic purpura is of a different order. Treatment of this disorder with vitamin C does not appear to have a rational basis.

Our findings fail to verify our original conception that an anatomic capillary lesion must be present before hemorrhage can occur. It is more likely that the passage of blood through the walls of the vessels is determined by the functional state of the capillaries, i.e., their degree of dilatation. This vasodilatation, when associated with a considerable platelet deficiency, may be the mechanism by which hemorrhage is produced. Capillary dilatation may be initiated by the rapid disintegration of platelets liberating some substances possibly of a histamine-like nature. We have no proof of this hypothesis, but experiments are under way to investigate the mechanism of capillary dilatation.

SUMMARY

An antiplatelet serum has been produced that is capable of selectively destroying blood platelets and causing purpura. The production of purpura is apparently related to the quantitative reduction in the number of circulating platelets and to the rapidity of the fall in the platelet level.

The effect of the serum on animals with anatomically intact capillaries and on animals with capillary lesions (vitamin C deficiency) is the same. This suggests that the capillary factor is functional rather than anatomic and could be more profitably studied by physiologic means. The hypothesis is advanced that capillary dilatation in addition to a platelet deficiency is necessary before hemorrhage can occur.

Vitamin C does not protect guinea pigs against experimentally produced thrombocytopenic purpura.

REFERENCES

1. Reese, H. M., and Ecker, E. E.: An Improved Method for Counting Blood Platelets, *J. A. M. A.* 80: 621, 1923.
2. Duke, W. W.: The Relation of Blood Platelets to Hemorrhagic Disease, *J. A. M. A.* 55: 1185, 1910.
3. Ledingham, J. C. G.: The Experimental Production of Purpura in Animals, *Lancet* 1: 1673, 1914.
4. Ledingham, J. C. G., and Bedson, S. P.: Experimental Purpura, *Lancet* 1: 311, 1915.
5. Julianelle, L. A., and Reimann, H. A.: Production of Purpura by Derivatives of Pneumococcus. 3. Further Studies on the Nature of the Purpura-Producing Principle, *J. Exper. Med.* 45: 609, 1927.
6. Freud, J.: Hemorrhages in Skin Lesions of Guinea Pigs Following Intravascular Injection of Toxins (Shwartzman Phenomenon), *J. Exper. Med.* 60: 661, 1934.
7. Tocantins, L. M.: Experimental Thrombopenic Purpura; Cytological and Physical Changes in the Blood, *Ann. Int. Med.* 9: 838, 1936.
8. Tocantins, L. M.: Experimental Thrombopenic Purpura in the Dog, *Arch. Path.* 21: 69, 1936.
9. Reimann, H. A., and Julianelle, L. A.: Production of Purpura by Derivatives of Pneumococcus; General Considerations of Reaction, *J. Exper. Med.* 43: 87, 1926.
10. Shih, H. E., and Jung, T. S.: Thrombocytopenic Purpura Hemorrhagica Produced Experimentally With Thorium, *Proc. Soc. Exper. Biol. & Med.* 29: 243, 1931.

11. Gottlieb, M. J.: *Experimental Purpura*, J. Immunol. 4: 309, 1919.
12. Lee, R. I., and Robertson, O. H.: The Effect of Antiplatelet Serum on Blood Platelets and the Experimental Production of Purpura Hemorrhagica, J. M. Research 33: 323, 1916.
13. Watabiki, T.: Studies on Experimental Purpura Hemorrhagica Produced by Anti-blood Platelet, Kitasato Arch. Exper. Med. 1: 195, 1917.
14. Roskam, J.: Purpuras hémorragiques et thrombopénie (Etude expérimentale). Pathogénie du syndrome hémogénique engendré par l'administration de sérum antiplaquettes, Sang 8: 129, 1934.
15. Bedson, S. P.: Blood Platelet Anti-Serum, Its Specificity and Role in Experimental Production of Purpura, J. Path. & Bact. 24: 469, 1921.
16. Archard, C., and Aynaud, M.: Action comparée de la peptone "in vivo" et "in vitro" sur les globulins, Compt. rend. Soc. de biol. 65: 554, 1908.
17. Chevreil and Roger: Isolement des hémoblastes—Production d'un serum anti-hémoblastique, Compt. rend. Soc. de biol. 63: 501, 1907.
18. Cole, R. I.: Note on the Production of an Agglutinating Serum for Blood Platelets, Bull. Johns Hopkins Hosp. 18: 261, 1907.
19. Bedson, S. P.: Blood Platelet Anti-Serum. Its Specificity and Role in the Experimental Production of Purpura, J. Path. & Bact. 25: 94, 1922.
20. Bedson, S. P.: Immunity to Anti-Platelet Serum, J. Path. & Bact. 26: 176, 1923.
21. Troland, C. E., and Lee, F. C.: Thrombocytopen: A Substance in the Extract From the Spleen of Patients With Idiopathic Thrombocytopenic Purpura That Reduces the Number of Blood Platelets, J. A. M. A. 111: 221, 1938.
22. Böger, A., and Schröder, H.: Ueber die Stillung schwerster Blutungen bei allen Formen der hämorrhagischen Diathese und der Hämophilie durch parenterale Zufuhr von C-vitamin ("ceviton Merk"), München. med. Wchnschr. 81: 133, 1934.
23. Böger, A., and Morton, W.: Vitamin C und Blut, München. med. Wchnschr. 82: 890, 1935.
24. Findlay, G. M.: The Blood and Blood-Vessels in Guinea Pig Scurvy, J. Path. & Bact. 24: 446, 1921.
25. Engelkes, H.: Treatment of Hemorrhagic Disorders With Vitamin C, Lancet 2: 1285, 1935.
26. Böger, A., and Schröder, H.: Vitamin C und Plasmaeiweisskörper, Klin. Wchnschr. 13: 842, 1934.
27. Junghaus, E.: Die Behandlung von gynäkologischen Blutungen mit Vitamin C, Klin. Wchnschr. 14: 899, 1935.
28. Vogt, E.: Ueber die Behandlung gynäkologischen Blutungen mit Vitamin C, München. med. Wchnschr. 82: 263, 1935.
29. Miller, D. K., and Rhoads, C. P.: Ascorbic Acid in the Treatment of Thrombocytopenic Purpura, J. Clin. Investigation 15: 462, 1936.
30. Fisher, B. H., and Leake, C. D.: The Parenteral Administration of Cevitamic Acid (Ascorbic Acid) Solutions, J. A. M. A. 103: 1556, 1934.
31. Winckelmann, H.: Zur Vitamin C Behandlung von hämorrhagischen Diathesen, Med. Klin. 34: 906, 1938.
32. Walther, G.: Misserfolge bei Vitamin C Therapie, Med. Klin. 34: 260, 1938.
33. Duryee, A. W., and Wright, I. S.: Studies of Human Capillaries; Present Day Technique for Study of the Human Capillaries, Am. J. M. Sc. 185: 664, 1933.
34. Schultz, P.: Studies on Capillary Resistance: Improbability of C Avitaminosis as Etiological Factor in Peptic Ulcer, Hospitalstid. 77: 1190, 1934.
35. Gothlen, G. F.: Outline of a Method for the Determination of the Strength of the Skin Capillaries and the Indirect Estimation of the Individual Vitamin C Standard, J. Lab. & Clin. Med. 18: 484, 1933.
36. Wright, I. S., and Lillienfeld, A.: Pharmacologic and Therapeutic Properties of Crystalline Vitamin C (Cevitamic Acid), With Especial Reference to Its Effects on Capillary Fragility, Arch. Int. Med. 57: 241, 1936.
37. Dalldorf, G.: A Sensitive Test for Sub-Clinical Scurvy in Man, Am. J. Dis. Child. 46: 794, 1933.
38. Wright, I. S.: The Clinical Value of Human Capillary Studies, J. A. M. A. 101: 439, 1933.
39. Wright, I. S., and Duryee, A. W.: Human Capillaries in Health and in Disease, Arch. Int. Med. 52: 545, 1933.
40. Cutter, I., and Marquardt, G.: Studies in Capillary Fragility, Proc. Soc. Exper. Biol. & Med. 28: 113, 1930.
41. Greene, D.: Evaluation of the Capillary Resistance Test in the Diagnosis of Sub-clinical Scurvy, J. A. M. A. 103: 4, 1934.
42. Flodorf, E. W., and Mudd, S.: Procedure and Apparatus for Preservation in "Lyophil" Form of Serum and Other Biological Substances, J. Immunol. 29: 389, 1935.

THE DEPRESSOR EFFECT OF POTASSIUM SULFOCYANATE BEFORE AND AFTER BILATERAL SPLANCHNICOTOMY IN NORMAL AND HYPERTENSIVE DOGS*

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IN ANOTHER paper we¹ have reported our experimental and clinical experiences with the administration of potassium sulfoeyanate to patients with essential hypertension and to dogs with hypertension produced by the Goldblatt ischemic method.

It has been shown that a certain number of patients with essential hypertension react favorably to the administration of potassium sulfoeyanate if the individual dosage is carefully controlled by blood cyanate level determinations. Accompanying the fall in the systolic and diastolic blood pressures, there is a decrease in the blood serum proteins; the blood cholesterol falls; the sedimentation rate increases markedly and the hematocrit reading decreases. These favorable reactions to the cyanates in patients have been confirmed in the experimental laboratory by administering the drug to normal and hypertensive dogs.

In another group of patients with essential hypertension, the response to the cyanates may be poor, and attempts to obtain a more favorable effect by increasing the level of the cyanates in the blood are followed by symptoms of cyanate toxicity which require that the administration of the drug be stopped.

We have also reported that after removal of the splanchnic nerves supradiaphragmatically in those patients with essential hypertension who have responded poorly to cyanate therapy, a certain number definitely react more favorably to the drug. The blood pressure levels, both systolic and diastolic, the blood cholesterol, serum proteins, hematocrit, and sedimentation rate accurately reflect this conversion to a satisfactory response.

As yet no one has been able to explain satisfactorily the effect of potassium sulfoeyanate in the treatment of essential hypertension. Claude Bernard and other earlier investigators believed that sulfoeyanate exerted its effect because it was a direct muscle poison and abolished muscular activity, a theory long since disproved. Westphal and Blum² suggested on purely theoretical grounds that cholesterol, as well as protein cleavage products, decreased the permeability of the cell membrane of the smooth muscles of the arterioles. Such a condition they believed, would favor an excessive retention of pressor substances in the cell normally present for the maintenance of tonus, and thus persistent arterial hypertonus would result. They further stated that the salt action of the cyanation might act upon the cholesterol at the cell membrane to produce an increase

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in the permeability of the arterial muscle cells, thus allowing an escape of the retained pressor substance which would result in a lowering of arterial tension. Although this explanation has never been accepted fully, some support is afforded by Gellhorn³ who observed that weak solutions of cyanate ion permeated the isolated striated muscle of a frog so slowly that no contraction occurred, but that when that same muscle containing cyanate ion was transferred to a cyanate-free solution, withdrawal of the ion was so rapid that actual contracture was produced. Thus he concluded that the ion had definitely increased the permeability of the muscle cell membrane.



Fig. 1.—Blood pressure response of a normal dog (weight, 17.2 kg.) to an intravenous injection of 0.25 Gm. of potassium sulfoeyanate.

In an effort to learn something of the method of action of potassium sulfoeyanate, a series of acute experiments were carried out upon six dogs with experimental ischemic hypertension, twelve normal dogs, and four normal dogs to which potassium sulfoeyanate had been administered over a sufficiently long period of time to establish a high blood cyanate level. In all of the hypertensive animals, in seven of the normal dogs, and in all of the normal dogs with blood cyanate levels, blood pressure tracings were taken before and after bilateral removal of 3 to 4 inches of the thoracic sympathetic trunks and ganglia above the diaphragm and section of the greater and lesser splanchnic nerves. Blood pressure tracings were made with a cannula in the carotid artery, and injections of potassium sulfoeyanate were made into the femoral vein. A tracheal cannula connected with an oxygen tank and an automatic respirator was used in each experiment and successfully combated the respiratory difficulties which attend opening the thoracic cavity bilaterally. In some of the early experiments a record of the outflow from the coronary artery was made.

The results of these acute experiments can be stated briefly because they were uniform when the dosage did not exceed the tolerance for the particular

dog under observation and when the reaction of the dog to the potassium ion in the drug did not produce a rise in the blood pressure and cardiac fibrillation which resulted in death. We were never able to predict which animal would exhibit these reactions. If the animal's tolerance was exceeded with the initial injection, death occurred promptly. That cardiac fibrillation was the reaction of the heart to the potassium ion in the drug, with a resulting sharp rise in the blood pressure, was proved in several animals by the injection of sodium sulfocyanate without such an effect. Occasionally, in some normal dogs the pressor effect of the potassium ion overshadowed the common depressor effect of the cyanates.

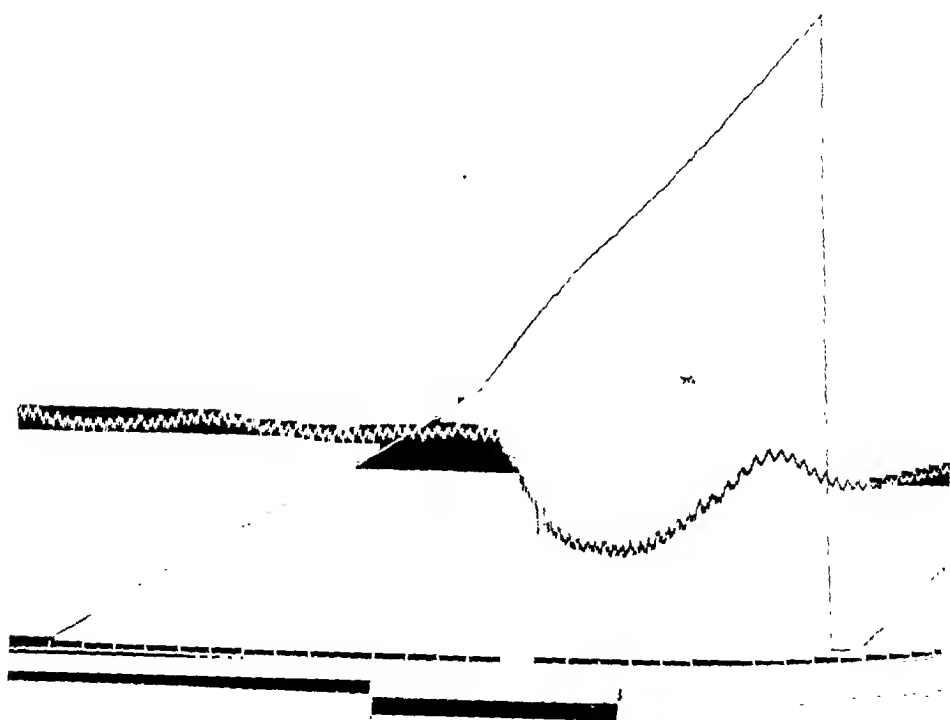


Fig. 2.—Blood pressure response and coronary outflow of normal dog (weight, 13 kg.) to intravenous injection of 0.50 Gm. of potassium sulfocyanate. Note sudden increase in coronary outflow, coincident with fall in pressure.

The usual prompt fall in blood pressure following injection of potassium sulfocyanate intravenously in small doses (0.013 Gm. per 1 kg.) makes it difficult to regard the effect as based upon anything other than a vasodilator mechanism (Fig. 1). The increased coronary outflow (Fig. 2) would seem to corroborate this view. Any explanation for the action of the cyanates as a protoplasmic toxic agent would not appear logical in the presence of such a prompt response. Fig. 3A illustrates the beginning of a blood pressure drop following an injection of potassium sulfocyanate which is quickly overtaken by the pressor action of the potassium ion and evidence of a beginning cardiac arrhythmia. Fig. 3B illustrates the more definite depressor response in this same animal to a similar dose of potassium sulfocyanate after the splanchnic nerves

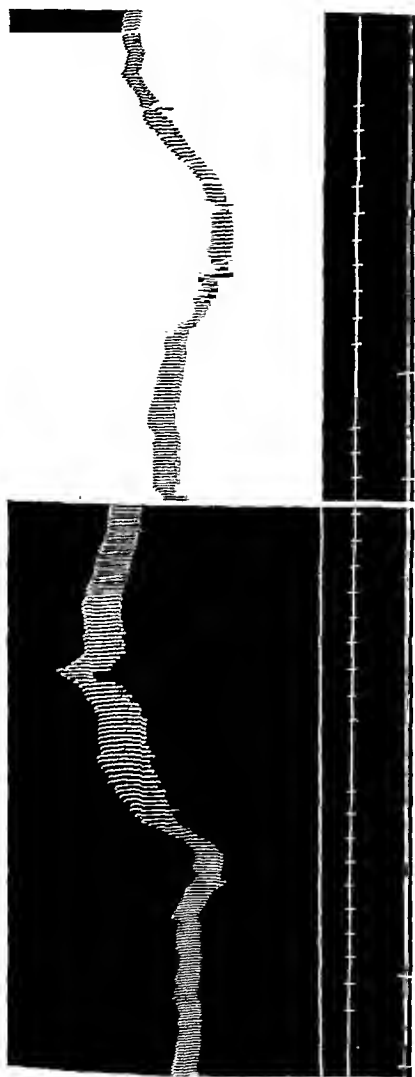


Fig. 3.—Blood pressure responses in normal dog (weight 18.2 kg.) to an intravenous injection of 0.50 Gm. of potassium sulfocyanate (A) before and (B) after supradiaphragmatic splancinctomy.

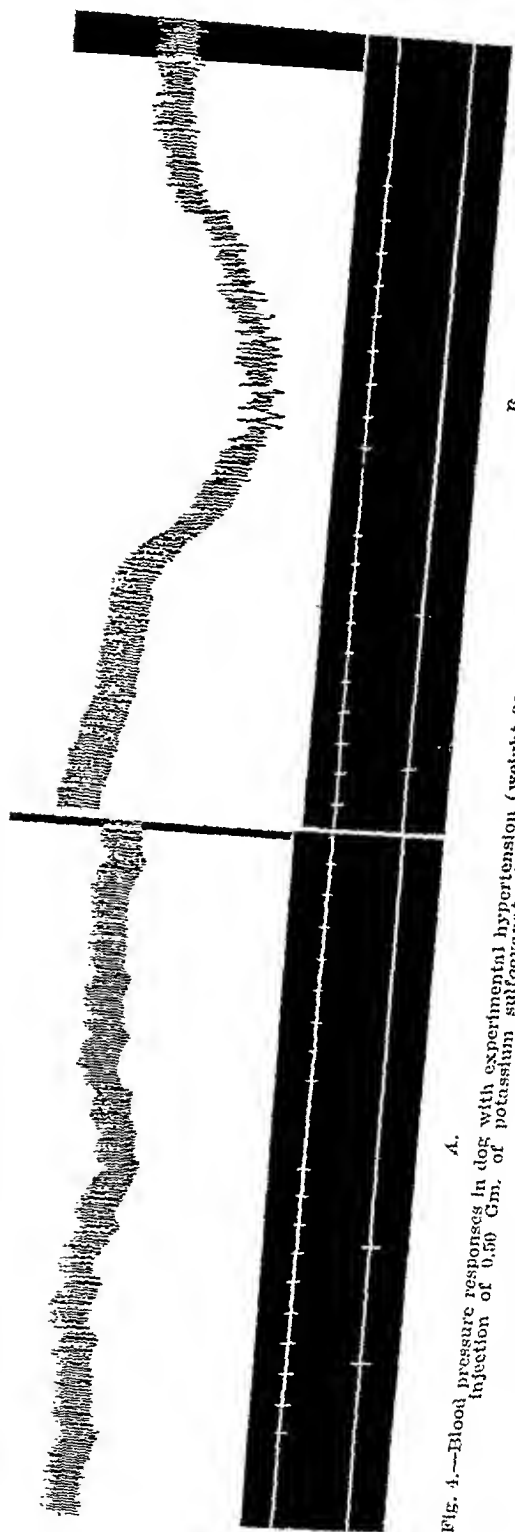


Fig. 4.—Blood pressure responses in dog with experimental hypertension (weight 20.9 kg.) and a blood cyanate level of 33 mg. to an intravenous injection of 0.50 Gm. of potassium sulfocyanate (*A*) before and (*B*) after supradiaphragmatic splenectomy.

were sectioned. This would seem to point definitely to the effect of the removal of the vasoconstrictor mechanism in the visceral area supplied by the splanchnics. Similar results were obtained upon dogs which had been given cyanates over a period of several days in order to establish a blood cyanate level. It was noted, however, that it required a smaller dose of the drug to produce a similar quantitative response in the blood pressure in these animals.

Fig. 4 (*A* and *B*) illustrates the response of the blood pressure in a hypertensive dog in which a partial ischemia of both kidneys had been produced several months previously by the application of Goldblatt clamps to the renal arteries. This particular animal had been fed potassium sulfo cyanate for several weeks and had been proved to be responsive to the drug, as evidenced by a fall in the mean arterial pressure and significant changes in the blood chemistry. Before this experiment was carried out, cyanate administration had been stopped and the blood pressure had returned to its previous high level. Fig. 4*B* shows the increased response after splanchnicotomy to the same dose of potassium sulfo cyanate as was injected in obtaining the tracing shown in Fig. 4*A*. Again the promptness of the response to injection of the cyanates speaks for a direct vasodilator action as against a general toxic depressor effect.

SUMMARY

From the results of acute experiments carried out upon normal dogs, ischemic hypertensive dogs, and normal dogs with elevated blood cyanate levels, it would seem logical to believe that the effect of the intravenous injection of potassium sulfo cyanate produces its depressor effect as the result of a general vasodilatation. This effect can be increased by removal of the thoracic sympathetic trunk and splanchnic nerves supradiaphragmatically. These experimental observations lend support to the observation made clinically that in many instances patients who respond poorly to cyanate therapy may become sensitive following a bilateral splanchnicotomy.

REFERENCES

1. Davis, Loyal, and Barker, M. H.: Clinical and Experimental Experiences in the Surgical Treatment of Hypertension, *Ann. Surg.* 110: 1016, 1939.
2. Westphal, K., and Blum, R.: Die Rhodanthérapie des genuinen arteriellen Hochdrucks und ihre theoretische Begründung, *Deutsches Arch. f. klin. Med.* 152: 331, 1926.
3. Gellhorn, E.: Further Experiments on Direct and Indirect SCN-Contracture; Dependence of Ion Antagonism on Permeability, *Am. J. Physiol.* 96: 447, 1931.

CLINICAL CHEMISTRY

THE ACID-BASE BALANCE AND WATER CONCENTRATION OF THE BLOOD DURING THE TOXEMIAS OF LATE PREGNANCY*

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A NUMBER of reports on the acid-base balance in the blood during the toxemias of late pregnancy have appeared recently.¹⁻⁵ Although there is some variation, depending upon the type and severity of the toxemia, it is generally agreed that the acid-base values are within the accepted range for normal pregnant women, except shortly after a convulsive seizure, when the bicarbonate and pH values are abnormally low, and during coma, when there is an acidosis caused by uncompensated alkali deficit (Van Slyke, area 9).

For the purpose of this study, the toxemias of late pregnancy are divided into three clinical groups: (1) chronic cardiovascular renal disease (essential hypertension, arteriolar sclerosis), (2) toxemia without convulsions, and (3) toxemia with convulsions (eclampsia). This classification has been employed in the clinic for some years and has proved to be satisfactorily simple. Patients representing each of these three groups were studied to determine the water concentration of the whole blood and the acid-base balance of the plasma by methods similar to those employed in investigating the blood changes in normal women during late pregnancy.^{6, 7}

METHODS AND MATERIALS

The analytical methods and necessary calculations were those described previously^{6, 7} in studies of normal pregnant women, similar precautions being observed during collection and analysis. Determinations included the water content and specific gravity of plasma and whole blood, the cell volume and hemoglobin content of whole blood, and the concentration of the important acidie and basic constituents of plasma.

RESULTS

Chronic Cardiovascular Renal Disease.—The clinical data on the nine patients in this group are presented in Table I; the blood chemical findings, in Tables I-A and I-B.

Comparison with the average values noted in the blood of normal pregnant women^{6, 7} shows little variation, although the hematocrit reading is somewhat elevated and the water in the cells is below normal. By reason of the depression of the chlorides, the average total acid is in the lower range of normal, as

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is the average total base, because of the diminished sodium content. Both variations may be explained by the lowered concentration of sodium chloride.

Toxemia Without Convulsions.—Data on the fifteen patients in this group are presented in Tables II, II-A, and II-B.

The water content of the plasma is slightly higher than in the hypertensive patients, but the cell water values are similar. The total bases and the total acids are both within the ranges found in normal pregnant women.

Toxemia With Convulsions.—Data on the seven patients with convulsive toxemia are presented in Tables III, III-A, and III-B.

The cell volume, the hemoglobin, the specific gravity of whole blood and plasma, and the water content of the plasma are within the ranges found in normal pregnant women, whereas the water content of the blood cells is significantly reduced.

The average values for total acids are in the lower range for normal pregnant women. Evidently the reduced acidity is caused by a diminution of the bicarbonate rather than by a lowering of the chloride concentration. This change more than compensates for the slight increase in the sulfates.

The average values for total base are slightly lower than in normal pregnant women, a finding which is contradictory to the observations of Stander, Eastman, Harrison, and Cadden.¹ The plasma pH values are generally lower than those for normal pregnant women, especially when the blood is drawn shortly after a convulsion.

DISCUSSION

The results generally confirm observations previously reported and support the contention of Kydd, Oard, and Peters² that changes in the acid-base balance of the blood in women suffering from the toxemias of late pregnancy are probably the result of the disease, and that therapy should logically be directed at the relief of the symptoms rather than at the correction of the slight, and possibly temporary, alkali deficit by the administration of alkalis.

Edema, which is a recognized component of the clinical syndrome of the late pregnancy toxemias, was manifest in the majority of the patients studied, although its extent varied markedly. The amount of edema appears to be unrelated to the water content of the plasma or whole blood, or to the plasma proteinate. This latter observation appears to be contrary to earlier work, which showed that the plasma protein concentration diminishes with increasing edema. Peters, Wakeman, Eisenman, and Lee³ have pointed out that the base-binding capacity of the total protein varies with the albumin-globulin ratio, becoming significantly less as the ratio diminishes. It is well recognized that in edema the diminution of the total plasma protein is largely caused by a reduction in albumin. Such a change would lower the ratio and consequently lead to a proteinate value which is too high. Assuming the correctness of this argument, the findings here recorded are in reasonable accord with previous observations. Moreover, it is obvious that if the recorded proteinate values are too high, the recorded values for total acids both in normal pregnant women and in those with toxemia would also be too high, and that the variations should be more marked in the latter group where the reduction of the A/G ratio is greater.

TABLE I
CHRONIC CARDIOVASCULAR RENAL DISEASE
Clinical Data

CASE NO.	HOSPITAL NO.	AGE	PREVIOUS PREGNANCIES	URINARY ALBUMIN	EDEMA	ADAMS- BLOOD PRESSURE (MM.)	RETINITIS	BLOOD NITROGEN RETEN- TION	METHOD OF DELIVERY	CHILD		REMARKS
										WEIGHT (GM.)	FATE	
18	G-410	22	0	+	Slight	235/140	Toxic	Slight Uric acid 6.2	Spontaneous, induced	480	Stillborn	Retinal edema and hemorrhages
22	G-645	43	6	+++	None	210/140	Arterio- sclerotic	None	Spontaneous, breach	500	Macerated	Generalized arterio- sclerosis
43a	E-6114	32	6	++	Slight	165/95	None	None	Spontaneous, induced	2,600	Survived	May, 1932
43b	E-6114	33	7	+++	Slight	250/150	None	None	Spontaneous	2,200	Survived	Two convulsions shortly after delivery, May, 1933
56	G-4593	32	2	+++	None	200/130	Early renal	None	Breach extraction	365	Macerated	Essential hypertension
60	G-4999	28	5	+	Slight	200/150	Hyper- tensive	None	Spontaneous	3,025	Survived	
74a	G-11423	45	7	++++	Slight	270/150	Toxic and arterio- sclerotic	None	Vaginal hysterot- omy	365	Stillborn	Admitted in coma; sev- eral convulsions 2 days before admission
74b												After 20 hours, medical treatment; slightly im- proved
74c												Two days later blood drawn shortly before de- livery. Patient died of puerperal septicemia on ninth postoperative day Patient not in hospital
79	Out- patient	?	1	++	None							
81	IL-1834	24	1	++++	Moder- ate	210/140	Renal	None	Cesarean hysterec- tomy	1,730	Survived	Detachment of retinae, essential hypertension
91	J-1409	23	0	+++	Slight	170/130	Neuro- retinitis	None	Spontaneous, induced	2,120	Survived	

TABLE I-A

CHRONIC CARDIOVASCULAR RENAL DISEASE
(Specific gravity, water and hemoglobin concentrations)

CASE NO.	CELL VOLUME (%)	SPECIFIC GRAVITY			WATER			HEMOGLOBIN		
		PLASMA	WHOLE BLOOD	CELLS	PER KG. PLASMA (GM.)	PER KG. WHOLE BLOOD (GM.)	PER KG. CELLS (GM.)	PER 100 C.C. WHOLE BLOOD (MM)	PER KG. CELLS (MM)	PER KG. WATER (MM)
18	40.0	1.0126	1.0388	1.078	922	812	654	8.26	19.15	29.30
22	37.8	1.0132	1.0323	1.062	900	810	668	8.93	21.88	32.80
43a	40.2	1.0199	1.0345	1.055	913	820	685	7.35	17.31	25.25
43b	37.2	1.0180	1.0441	1.087	920	818	665	7.30	17.90	26.90
56	51.0	1.0158	1.0507	1.083	912	776	654	9.41	17.02	26.02
60	39.5	1.0167	1.0411	1.075	912	807	656	8.17	19.24	29.28
74a	52.0	1.0197	1.0507	1.081	908	778	664	9.95	17.70	26.63
74b	43.0	1.0158	1.0443	1.082	932	810	672	8.22	17.66	26.30
74c	41.0	1.0171	1.0436	1.081	921	814	668	8.14	18.36	27.46
79	44.5	1.0175	1.0437	1.088	901	786	651	9.15	18.90	29.00
81	46.0	1.0180	1.0412	1.069	916	793	657	8.68	17.70	26.06
91	42.7	1.0126	1.0427	1.080	918	798	648	9.50	20.60	31.75
Avg.	42.9	1.0166	1.0423	1.077	915	802	662	8.59	18.62	28.14
Normal pregnant	35.4	1.0154	1.0370	1.078	915	827	674	7.00	18.31	27.23

TABLE I-B

CHRONIC CARDIOVASCULAR RENAL DISEASE

Acid-Base Equilibria in Plasma

(Concentrations are reported in terms of milliequivalents per kilogram of water.)

CASE NO.	[Cl] ⁻	[HCO ₃] ⁻	[PRO-TEINATE] ⁻	[H ₂ PO ₄] ⁻	[SO ₄] ⁻	TOTAL ACIDS	[Na] ⁺	[Ca] ⁺⁺	[K] ⁺	[Mg] ⁺⁺	TOTAL BASE	pH
18	108.6	20.2	12.8	2.17	0.5	144.3	145.3	4.37	5.48	2.3	157.5	7.26
22	107.8	28.3	18.1	2.74	1.3	158.2	151.2	5.10	5.14	2.0	163.4	7.43
43a	108.6	26.3	14.6	2.01	0.3	151.8	153.7	4.87	4.67	2.0	163.4	7.38
43b												
56	108.0	28.7	15.3	2.12	0.9	155.0	150.8	4.80	4.28	2.0	161.9	7.39
60	108.5	25.2	18.3	2.56	0.9	155.5	148.9	6.35	4.26	2.0	161.5	7.38
74a	104.8	24.6	14.7	2.32	0.7	147.1	151.9	4.97	5.61	1.9	164.4	7.19
74b	102.1	25.2	13.1	2.51	0.7	143.6	144.1	4.75	5.12	2.1	156.1	7.36
74c	104.2	25.5	13.7	1.89	0.7	146.0	149.1	5.02	6.33	2.2	162.6	7.32
79	112.0	26.3	17.3	1.92	0.8	158.3	156.7	5.66	5.78	2.2	170.4	7.48
81	106.0	28.4	17.8	2.98		156.0*	144.8	4.66	5.12	2.4	157.0	7.48
91	109.6	22.8	14.2	1.97		149.4*	155.1	4.50	7.65	2.3	170.0	7.44†
Avg.	107.3	25.6	15.4	2.29	0.8	151.4	150.1	5.01	5.40	2.13	162.6	7.37
Normal pregnant	112.2	24.6	15.8	2.35	0.55	155.5	151.6	5.15	5.10	2.35	164.1	7.39

*A value of 0.8 was assumed for [SO₄]⁻ --

†Determined colorimetrically and corrected to electrometric values by subtracting a determined value of 0.34.

The average values for plasma pH show no marked variation, but there is a significant diminution in those women with convulsive toxemia (eclampsia). Tables III and III-B show that the lowest values were obtained shortly after convulsions. For example, in Case 80, where the blood sample was drawn three minutes after the last (seventh) convulsion, the pH was 7.04, whereas in Case 17 b, a value of 7.22 was obtained several hours after the fifth attack, while the patient was still comatose. In these instances the low pH was associated with

TABLE II
TOXEMIA WITHOUT CONVULSIONS

CASE NO.	HOSPITAL NO.	AGE (YEARS)	PREVIOUS PREG-NANCIES	URINARY ALBUMIN	EDEMA	ADMISSION BLOOD PRESSURE (MM.)	RETINITIS	BLOOD NITROGEN RETENTION	METHOD OF DELIVERY	CHILD		REMARKS
										WEIGHT (GM.)	FATE	
23	G-997	26	0	++++	0	175/105	None	None	Spontaneous	1,450	Stillborn	
28	F-8751	32	0	+	Slight	170/110		None	Vaginal cesarean	1,940	Survived	Hemiatrophy of body
34	G-2223	30	4	+	Moderate	135/90			Spontaneous	3,640	Survived	Mild toxemia
46	G-3676	41	1	++	Moderate	170/100	None	None	Spontaneous	1,545	Survived	
48	G-2686	16	0	+	Slight	170/100			Spontaneous	3,900	Survived	
49	G-3929	36	4	++++	Slight	200/110	None	None	Spontaneous	2,645	Survived	
67	G-6109	40	5	+	Marked	180/90	None		Spontaneous, induced	2,175	Stillborn	Marked edema of fetus and placenta
72	G-10567	33	0	+++	Moderate	160/100	None		Cesarean section	2,220	Survived	Marked edema of vulva
73	G-11278	30	1	+++	Slight	200/140	None	Uric acid 5.4 mg.	Spontaneous	2,400	Died on third day	
77a	G-11913	21	0	++++	Moderate	155/115	None	None	Spontaneous	2,640	Macerated	One convulsion 3 days later (see 77b on Table III)
83	II-3412	17	0	++++	Marked	148/100	None	Uric acid 8.4 mg.	Cesarean section	1,560 1,600	Both died on first day	Marked edema of vulva
89	D-5247	25	0	+++	Slight	165/115		None	Spontaneous, induced	2,175	Survived	
90	II-13800	32	3	+	Marked	180/95		None	Spontaneous, induced	2,990	Survived	Hemolytic icterus
95	J-6209	33	9	+++	Moderate	240/140	None	None	Brechi extraction, induced	1,550	Died on first day	Hypertension persisted after delivery. Chronic nephritis?
96	J-6504	34	2	+++	Marked	195/100	None	None	Spontaneous, induced	735	Stillborn	Hyperticroidism, anencephalic monster

TABLE II-A

TOXEMIA WITHOUT CONVULSIONS

(Specific gravity, water and hemoglobin concentrations)

CASE NO.	CELL VOLUME (%)	SPECIFIC GRAVITY			WATER			HEMOGLOBIN		
		PLASMA	WHOLE BLOOD	CELLS	PER KG. PLASMA (GM.)	PER KG. WHOLE BLOOD (GM.)	PER KG. CELLS (GM.)	PER 100 C.C. WHOLE BLOOD (MM)	PER KG. CELLS (MM)	PER KG. WATER (MM)
23	38.0	1.0100	1.0410	1.093	914	808	648	8.34	20.08	31.20
28	35.0	1.0165	1.0330	1.058	908	820	664	7.81	21.10	31.80
34	39.2	1.0078	1.0395	1.089	910	806	657	9.02	21.12	32.20
46	38.5	1.0095	1.0244	1.048	927	826	672	7.85	19.45	28.95
48	32.0	1.0142	1.0345	1.079	924	844	686	6.20	17.95	26.17
49	26.5	1.0149	1.0352	1.091	924	842	628	5.98	20.65	32.88
67	35.8	1.0122	1.0383	1.086	930	835	669	6.65	17.10	25.58
72	42.0	1.0158	1.0416	1.078	920	807	658	8.46	18.70	28.40
73	44.0	1.0120	1.0431	1.068	927	793	633	8.54	18.20	27.10
77a	41.5	1.0130	1.0410	1.081	914	810	670	8.44	18.80	28.10
83	36.7	1.0098	1.0389	1.090	928	827	662	7.08	17.70	26.70
89	42.0	1.0192	1.0446	1.080	914	801	655	8.82	19.42	29.70
90	27.5	1.0184	1.0366	1.086	910	855	713	4.25	14.22	20.00
95	46.0	1.0121	1.0459	1.088	908	783	641	10.12	20.21	31.55
96	29.0	1.0125	1.0291	1.065	928	854	681	6.12	19.75	29.00
Avg.	36.9	1.0132	1.0378	1.079	919	821	662	7.59	18.96	28.62
Normal pregnant	35.4	1.0154	1.0370	1.078	915	827	674	7.00	18.31	27.23

TABLE II-B

TOXEMIA WITHOUT CONVULSIONS

Acid-Base Equilibria in Plasma

(Concentrations are reported in terms of milliequivalents per kilogram of water.)

CASE NO.	[Cl] ⁻	[HCO ₃] ⁻	[PRO-TEINATE] ⁻	[HPO ₄] ⁻ + [H ₂ PO ₄] ⁻	[SO ₄] ⁻	TOTAL ACIDS	[Na] ⁺	[Ca] ⁺⁺	[K] ⁺	[Mg] ⁺⁺	TOTAL BASE	pH
23	109.6	28.0	16.4	2.47	0.4	156.9	149.5	5.50	4.71	2.2	161.9	7.24
28	109.7	24.1	16.7	2.56	0.6	153.7	144.5	4.26	6.36	2.6	157.7	7.42
34	113.6	23.7	16.6	2.43	0.4	156.7	167.3†	6.43	4.76	2.5	181.0†	7.37
46	113.0	22.7	14.8	2.47	0.3	153.3	147.7	4.98	4.90	1.9	159.5	7.26
48	112.2	24.6	16.5	2.26	0.9	156.5	156.4	4.98	5.44	1.9	168.7	7.30
49	116.4	24.3	16.0	2.64	0.2	159.5	156.7	5.42	5.03	2.5	169.7	7.30
67	117.9	18.5	16.0	2.95	1.5	156.9	147.0	5.39	5.72	1.9	160.0	7.35
72	111.4	21.9	16.4	2.82	0.7	153.2	161.2	5.00	6.28	1.9	174.4	7.42
73	108.2	27.0	15.6	2.50	1.0	154.3	152.7	5.00	5.18	2.1	165.0	7.37
77a	112.8	23.9	15.2	2.63	0.7	155.2	158.8	5.19	5.60	2.0	171.6	7.41
83	108.8	17.3	17.2	3.10		147.2†	146.0	4.64	7.26	2.3	160.2	7.28
89	111.1	23.3	14.2	2.56		152.0†	147.6	4.60	5.34		159.7*	7.16†
90	114.0	18.5	16.6	2.27		152.2†	151.0	5.06	5.45		163.7*	7.28†
95	102.2	26.6	18.1	3.89		151.6†	146.9	5.16				7.44†
96			15.9									
Avg.	111.5	23.2	16.1	2.68	0.7	154.3	151.2	5.12	5.54	2.2	164.3	7.33
Normal pregnant	112.2	24.6	15.8	2.35	0.55	155.5	151.6	5.15	5.10	2.35	164.1	7.39

*Determined colorimetrically and corrected to electrometric values by subtracting a determined value of 0.34.

†A value of 2.2 was assumed for [Mg]⁺⁺.‡A value of 0.8 was assumed for [SO₄]⁻.

§Not included in the average.

TABLE III
TOXEMIAS WITH CONVULSIONS

CASE NO.	HOSPITAL NO.	AGE	PARITY	URINARY ALBUMIN	EDEMA	ADMIS- SION BLOOD PRES- SURE (mm.)	RETINITIS	BLOOD NITROGEN RETENTION	METHOD OF DELIVERY	CHILD		REMARKS
										WEIGHT (gm.)	FATE	
17a	G-23	20	0	+++	Slight	166/130†	Retinal edema only	None; uric acid 5.7	Low forceps and epis- iotomy	2700	Survived	Blood drawn 2 hours after the fifth con- vulsion. Patient undelivered
17b					Slight	155/108*						Blood drawn 12 hours, after deliv- ery. Patient in coma; no more convulsions
44	G-3443	18	0	++	Slight	170/90	Normal fundus	None; uric acid 4.5	Spontaneous	1850	Stillborn	One convulsion. Premature separa- tion of the pla- centa. Acquired syphilis, latent
77b	G-11913	21	0	+++	Moderate	185/120	Normal fundus	None	Spontaneous	2640	Stillborn, macerated	One convulsion 45 minutes before blood was drawn

80	Out-patient case†	?	0	+	Moderate	170/110	?	?	?	?	?	One convulsion before and six after delivery. Blood drawn 3 minutes after last convulsion
84	Out-patient case†	?	2	++++	Moderate	166/108	?	?	?	?	?	Two convulsions. Blood drawn 12 hours after second
92	J-5215	21	2	+	Slight	180/100	Normal fundi	No note	Spontaneous	3518	Survived	One post-partum convulsion. Blood drawn 4 hours later
93	J-5370	42	3	++++	Slight	160/100	Small retinal vessels show hy-per-tension. Right retina edematous	Slight; N. P. N. 46 mg.	Spontaneous	850	Stillborn, macerated	Seven convulsions. Blood drawn 1½ hours after last convulsion. Cyanotic with stertorous respiration

*After venesection of 140 c.c. (20 min. later) blood pressure was 140/95. 40 min. later it was 138/90.

†Blood pressure was 125/80 after the convulsion, 30 gr. chloral hydrate was given by rectum after fourth convulsion. One and one-half hours later blood pressure was 142/110; patient was irrational; then ½ hr. later the blood was drawn, and 50 c.c. of 50 per cent glucose were given intravenously. Patient soon became rational.

‡Blood collected in out-of-town hospitals. Little clinical data available.

TABLE III-A
TOXEMIA WITH CONVULSIONS
(Specific gravity, water and hemoglobin concentrations)

CASE NO.	CELL VOLUME (%)	SPECIFIC GRAVITY			WATER			HEMOGLOBIN		
		PLASMA	WHOLE BLOOD	CELLS	PER KG. PLASMA (GM.)	PER KG. WHOLE BLOOD (GM.)	PER KG. CELLS (GM.)	PER 100 C.C. WHOLE BLOOD (MM.)	PER KG. CELLS (MM.)	PER KG. WATER (MM.)
17a	31.8									
17b	34.2	1.0128	1.0296	1.056	913	817	636	6.61	18.31	28.78
44	38.5	1.0141	1.0377	1.074	913	817	672	7.14	17.22	25.63
77b	38.2	1.0068	1.0359	1.081	915	813	659	7.70	18.62	28.30
80	39.9	1.0109	1.0388	1.080	912	806	656	8.11	18.80	28.60
84	39.0	1.0144	1.0297	1.053	918	822	677	8.48	20.60	30.40
92	31.5	1.0170	1.0377	1.085	916	835	666	6.38	18.65	28.00
93	41.0	1.0160	1.0459	1.090	918	801	645	9.88	19.85	30.80
Avg.	37.5	1.0133	1.0364	1.074	915	816	659	7.76	18.86	28.60
Normal pregnant	35.4	1.0154	1.0370	1.078	915	827	674	7.00	18.31	27.23

TABLE III-B
TOXEMIA WITH CONVULSIONS
Acid-Base Equilibria in Plasma

(Concentrations are reported in terms of milliequivalents per kilogram of water.)

CASE NO.	[Cl] ⁻	[HCO ₃] ⁻	[PROTEIN-ATP] ⁻	[HPO ₄] ⁻ + [H ₂ PO ₄] ⁻	[SO ₄] ⁻	TOTAL ACIDS	[Na] ⁺	[Ca] ⁺⁺	[K] ⁺	[Mg] ⁺⁺	TOTAL BASE	pH
17a*	115.5		14.2	2.50	2.6		142.5	3.70	5.38	2.7	151.1	7.19
17b	115.7	15.3	13.5	1.78	2.6	148.0	141.8	3.76	5.51	2.7	155.8	7.22
41	107.0	23.2	18.5	2.18	0.2	151.1	156.1	5.10	4.69	2.0	165.5	7.34
77b	112.2	18.2	13.1	2.91	1.5	147.9	155.9	4.89	5.46	2.1	168.7	7.27
80	119.5	14.4	11.7	3.30	0.4	149.3	162.1	5.31	5.63	2.4	175.4	7.01
84			18.1				157.2	5.92				
92	105.8	27.0	19.2	2.60		155.14	110.0	1.65	8.74	2.1	155.8	7.44†
93	106.9	23.6	17.1	2.22		149.54	133.8	4.56	5.61	2.3	166.3	7.38†
Avg.	111.8	20.1	15.7	2.51	1.5	150.5	119.0	1.51	5.80	2.1	163.2	7.26
Normal pregnant	112.2	21.0	15.8	2.35	0.55	155.5	151.6	5.15	5.10	2.35	161.1	7.39

*Concentrations were calculated on the basis of the water content of the blood in 17b.

†Determined colorimetrically and corrected to electrometric values by subtracting a determined value of 0.31

‡A value of 0.8 was assumed for [SO₄]⁻.

a marked reduction of the bicarbonate. While no determinations for lactic acid were made, it seems probable that these observed changes toward the acid side were due to an accumulation of lactic acid developed in the organism as a result of the convulsive seizures, as emphasized by Stander.^{9, 10}

SUMMARY

The acid-base balance in the blood has been studied in thirty-one women suffering from various forms of the toxemia of late pregnancy (nine hypertensive, fifteen nonconvulsive, and seven eclamptic patients). In general, the findings confirm the work of previous investigators by demonstrating a slight alkali deficit, without any significant change in the hydrogen-ion concentration, except in eclampsia, where a convulsive seizure is followed by a marked reduction in the bicarbonate and an associated, proportional lowering of the pH.

On the basis of accumulated evidence, it would appear that the altered acid-base relationships of the blood in patients with pregnancy toxemia are of minor importance, and that any serious metabolic disturbances accompanying this disease entity must be sought in some tissue other than the blood.

REFERENCES

1. Stander, H. J., Eastman, N. J., Harrison, E. P. H., Jr., and Cadden, J. F.: *The Acid-Base Equilibrium of the Blood in Eclampsia*, J. Biol. Chem. 85: 233, 1929.
2. Stander, H. J., Eastman, N. J., and Harrison, E. P. H., Jr.: *The Acid-Base Equilibrium of the Blood in the Late Toxemias of Pregnancy*, Am. J. Obst. & Gynec. 19: 26, 1930.
3. Stander, H. J., and Eastman, N. J.: *The Hydrogen-Ion Concentration of the Blood in Eclampsia*, Am. J. Obst. & Gynec. 20: S22, 1930.
4. Muntwyler, E., Limbach, N., Bill, A. H., and Myers, V. C.: *The Acid-Base Equilibrium of the Blood in Pathological Conditions. I. Changes Observed in the Toxemias of Pregnancy*, J. Biol. Chem. 90: 607, 1931.
5. Kydd, D. M., Oard, H. C., and Peters, J. P.: *The Acid-Base Equilibrium in Abnormal Pregnancy*, J. Biol. Chem. 98: 241, 1932.
6. Oberst, F. W., and Plass, E. D.: *Water Concentration of the Blood During Pregnancy, Labor, and the Puerperium*, Am. J. Obst. & Gynec. 31: 61, 1936.
7. Oberst, F. W., and Plass, E. D.: *The Acid-Base Balance in the Plasma and Blood Cells of Normal Nonpregnant, Pregnant, and Puerperal Women*, J. LAB. & CLIN. MED. 26: 513, 1940.
8. Peters, J. P., Wakeman, A. M., Eisenman, A. J., and Lee, C.: *Total Acid Base Equilibrium of Plasma in Health and Disease. X. The Acidosis of Nephritis*, J. Clin. Investigation 6: 517, 1929.
9. Stander, H. J.: *Studies in Anesthesia, Anoxemia, Anhydremia and Eclampsia, With Certain Deductions Concerning the Treatment of Eclampsia*, Am. J. Obst. & Gynec. 12: 633, 1926.
10. Stander, H. J.: *Clinical and Experimental Studies on the Toxemias of Pregnancy*, Am. J. Obst. & Gynec. 13: 551, 1926.

EFFECT OF DIET ON ARTERIAL AND VENOUS GLUCOSE TOLERANCE CURVES IN RHEUMATOID ARTHRITIS*

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CHRONIC rheumatism is the center of considerable investigative work since it is one of the chief diseases causing partial or total disability in man. Rheumatoid arthritis is a type of chronic arthritis which is especially important economically since it attacks the young adult, as a rule, and converts an economic asset into a liability.¹⁻⁴ Considerable investigative work has been done in an effort to explain the causes, as well as changes, that occur in the body of the patient afflicted with this disease. The possibility of some derangement in the metabolic or endocrine system of the body has been considered as a possible etiologic factor;⁵⁻⁸ however, chronic infection seems to interest the greatest number of investigators.¹¹⁻¹³

Regardless of etiology, certain alterations in body economy are said to exist in the rheumatoid types of arthritis; there is a loss of muscle bulk, diminished circulation, and gastrointestinal stasis. Certain alterations in the chemistry of the body are also said to occur. A diminution in the blood content of vitamins B and C has been reported,^{9, 10} and there is said to be a diminished tolerance for glucose.¹⁴

Pemberton and others have shown that the arthritic person does not remove glucose from the blood as promptly as the normal individual. Because of the apparent reduction in carbohydrate metabolism, many investigators recommend low carbohydrate diets for the rheumatoid arthritic patient and have reported marked relief from pain when patients are placed on a high fat, low carbohydrate diet.

The experience of our clinic has been similar to that of Pemberton and others, in that the majority of the patients studied had a decreased tolerance for dextrose. We have felt that this diminished tolerance might also be explained by an impairment in the glycogenic function of the liver. If this is the case, improvement in the carbohydrate metabolism would be expected with a high carbohydrate rather than a high fat diet. Since glucose tolerance tests done on venous blood give only an idea of the blood sugar after the blood has coursed through the muscles, it was thought that a better idea regarding the ability of the liver to remove glucose from the blood would be obtained by the simultaneous estimation of both arterial and venous blood. In this way the level of the blood sugar could be ascertained after the blood has passed through the

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liver but not through the muscles. If the arterial blood sugar is high, the ability of the liver to remove sugar from the blood is impaired. On the other hand, if both the venous and arterial blood sugars are high and tend to approximate each other, the glycogenic function of both liver and muscle is impaired, since, when muscle function is good, the arteriovenous difference should be marked. It has been reported by Sweeney, and confirmed by others,¹⁶ that a high carbohydrate diet improves carbohydrate utilization in normal individuals. It was felt that it would be of value to determine whether: (1) diet had the same effect on the glucose tolerance of arthritic patients as that of normal individuals; (2) improvement followed one of the two diets used, whether it was due to improved liver or muscle function; (3) what the effect of the respective diets on the well-being of the patient might be.

PROCEDURE

Patients with typical rheumatoid arthritis were selected for the study with each patient showing varying degrees of muscle wasting and joint involvement of from two to twelve years' duration. Each patient was carefully examined for foci of infection. Blood nonprotein nitrogen, sugar, cholesterol, uric acid, Wassermann, and gonococcal complement fixation determinations were studied. Complete blood counts were done and chest x-ray studies were made.

Eleven patients, eight males and three females, were found to be suitable for this study; all had normal values for the above determinations. Each patient remained afebrile during the study and only occasionally received codeine with salicylates as symptomatic treatment. Three normal subjects, two females and one male, were also selected for the same study and acted as controls. Those selected were hospitalized and placed first on a high fat, low carbohydrate diet for two weeks; then on a high carbohydrate, low fat diet for another period of two weeks. At the end of this period the patient remained on a high carbohydrate diet but was given 10 units of insulin before each meal for a third period of two weeks. At the end of each period complete arteriovenous glucose tolerance tests were run on each patient, the arterial and venous bloods being collected at the same time. The various diets were equicaloric and contained an equal amount of protein. The high carbohydrate diet contained from 350 to 450 Gm. of carbohydrate and from 40 to 50 Gm. of fat, while the high fat diet contained from 200 to 225 Gm. of fat, and from 70 to 80 Gm. of carbohydrates.

The glucose tolerance test was run over a three-hour period. Following the collection of the fasting specimen of blood, a solution containing 100 Gm. of dextrose was taken by mouth. Specimens of blood were obtained from the cubital vein after thirty minutes, sixty minutes, two hours, and three hours. The arterial blood was collected from the finger tip simultaneously with the venous puncture, since it has been shown that cutaneous and arterial blood are identical in sugar values.¹⁷

RESULTS

Table I represents the results obtained in the three normal individuals. It will be noted that a lowering in the blood sugar curves occurred following a high carbohydrate diet. The glucose tolerance curve was more elevated after

the patient had been given insulin, plus a high carbohydrate diet, than it was when the patient was on a high carbohydrate diet, but the elevation was less than when the high fat diet was taken.

In the patients listed in Table II only venous sugars were obtained. It will be noted that the venous levels following a high fat diet reached almost diabetic proportions; however, following a high carbohydrate diet, the curves were considerably flattened.

TABLE I

PATIENT	DIET	MG. % SUGAR	TIME—MINUTES					AVG. DIFF. AT 30 MIN. (MG. %)
			FASTING	30	60	120	180	
M. M. F—20 yr.	High fat	Arterial	80	200	142	78	124	35
		Venous	76	165	118	69	114	
	Low fat	Arterial	83	128	106	69	101	15
		Venous	74	113	88	58	85	
	After insulin	Arterial	94	185	99	117	106	45
		Venous	81	140	88	98	89	
P. G. F—21 yr.	High fat	Arterial	70	145	156	115	50	46
		Venous	69	103	109	87	49	
	Low fat	Arterial	80	151	156	106	110	45
		Venous	73	121	111	74	80	
	After insulin	Arterial	85	170	164	115	106	32
		Venous	73	138	134	94	84	
R. A. M—28 yr.	High fat	Arterial	101	133	138	101	83	52
		Venous	80	116	85	85	76	
	Low fat	Arterial	85	112	106	100	88	26
		Venous	82	110	80	87	78	
	After insulin	Arterial	82	140	110	110	100	17
		Venous	76	123	105	96	80	

TABLE II

TIME	R. L. R. 32 YR.—FEMALE			J. L. B. 37 YR.—MALE			R. R. B. 28 YR.—MALE		
	HIGH FAT	LOW FAT	AFTER INSULIN	HIGH FAT	LOW FAT	AFTER INSULIN	HIGH FAT	LOW FAT	AFTER INSULIN
	VENOUS BLOOD MG. %			VENOUS BLOOD MG. %			VENOUS BLOOD MG. %		
Fasting	99	96	87	85	75	71	103	81	81
30	187	185	147	151	107	154	183	112	134
60	208	129	223	170	160	165	188	134	156
120	229	118	171	76	71	134	189	103	125
180	187	75	112	52	64	113	132	67	61

Both arterial and venous blood sugars were determined simultaneously in the eight patients listed in Table III. The tolerance is much diminished following a high fat diet, but is improved following a high carbohydrate diet. The effect of insulin is the same as in the normal individuals and points to a definite diminution in tolerance. Comparison of the arterial and venous blood sugars reveals a definite flattening of both the arterial and venous curves following a high carbohydrate diet, with a greater effect on the arterial curve. In four of the eight patients there occurred an increase in the arteriovenous difference following the use of a high fat diet.

Chart I was obtained by plotting the average values of both arterial and venous blood sugars so that an idea of the effect of diet and insulin might be ob-

TABLE III

PATIENT	DIET	MG. SUGAR	TIME—MINUTES					AVG. DIFF.
			FASTING	30	60	120	180	
H. R. 31 yr. Male	High fat	Arterial	95	200	210	140	128	33
		Venous	92	154	169	107	94	
	Low fat	Arterial	97	160	180	160	130	35
		Venous	89	140	138	105	89	
	Insulin	Arterial	108	148	162	169	98	24
		Venous	79	138	134	121	79	
V. B. 36 yr. Male	High fat	Arterial	108	142	183	144	97	22
		Venous	73	134	145	125	95	
	Low fat	Arterial	86	132	124	116	85	9
		Venous	82	128	118	100	80	
	Insulin	Arterial	83	156	186	164	99	9
		Venous	78	154	175	151	97	
C. M. 29 yr. Male	High fat	Arterial	75	178	187	142	118	23
		Venous	76	158	141	109	105	
	Low fat	Arterial	88	138	170	138	116	24
		Venous	83	131	134	103	101	
	Insulin	Arterial	74	144	192	168	160	25
		Venous	71	123	156	154	128	
A. M. 28 yr. Male	High fat	Arterial	88	218	227	174	128	54
		Venous	78	154	156	123	98	
	Low fat	Arterial	97	151	135	88	78	22
		Venous	88	114	107	71	71	
	Insulin	Arterial	88	192	196	192	119	25
		Venous	80	174	158	158	92	
N. B. 28 yr. Male	High fat	Arterial	90	186	210	140	122	33
		Venous	80	140	156	120	110	
	Low fat	Arterial	95	174	138	128	122	23
		Venous	80	143	100	99	88	
	Insulin	Arterial	92	186	169	115	98	20
		Venous	91	158	145	100	85	
R. H. 27 yr. Female	High fat	Arterial	75	144	220	219	192	15
		Venous	71	138	190	194	185	
	Low fat	Arterial	78	180	200	164	140	17
		Venous	75	158	188	150	118	
	Insulin	Arterial	90	148	215	210	189	12
		Venous	88	140	200	194	180	
S. J. 32 yr. Male	High fat	Arterial	88	188	221	193	162	31
		Venous	81	158	190	180	112	
	Low fat	Arterial	89	145	123	109	95	7
		Venous	85	138	118	100	92	
R. J. 26 yr. Female	High fat	Arterial	75	142	190	180	147	18
		Venous	71	129	180	173	124	
	Low fat	Arterial	88	167	189	94	98	10
		Venous	86	161	179	83	88	

tained at a glance. The flattening of both arterial and venous curves in the normal individual following the high carbohydrate diet is striking. Following insulin, the curve resembles that obtained by a high fat diet. It will be noted that the arthritic person tends to have a higher blood sugar following a carbohydrate test meal than does the normal individual; nevertheless, the response to a high carbohydrate diet, as well as to insulin, is similar to the effect produced in the normal individual. Following a high carbohydrate diet, the arterial curve of the arthritic person approaches that of the normal, indicating an improved utilization of sugar by the liver.

DISCUSSION

The result of our work demonstrates that a diminished tolerance for carbohydrates exists in rheumatoid arthritis, as was reported by Pemberton and

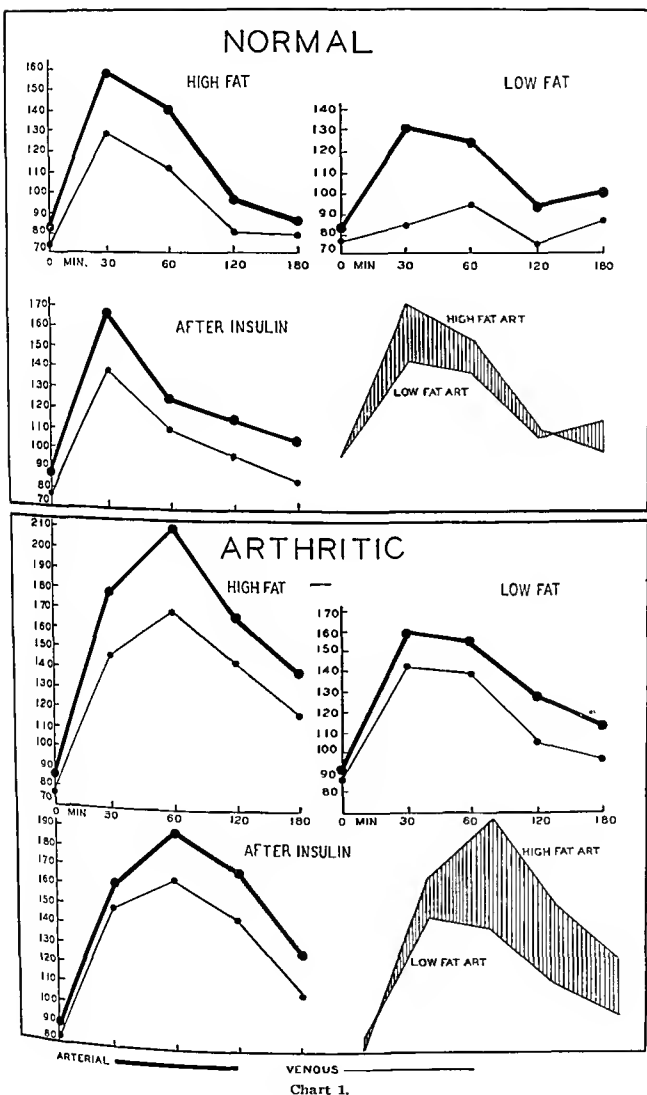


Chart 1.

others. Following a two weeks' course of insulin, these patients have a decreased tolerance to glucose, as well as a diminution in arteriovenous difference similar to those exhibited by our normal controls. This only adds to common knowledge that insulin is diabetogenic when given to a person without diabetes over a long period of time. Various explanations have been offered to account for the diminished tolerance to glucose: (a) disturbance of pancreatic function, (b) diminished circulation causing a slowing in the removal of sugar from the blood, and (c) hepatic dysfunction. From the evidence submitted, it would seem that the most important role is played by the liver. This is shown by (1) the improvement following the high carbohydrate diet, (2) a decrease in arterial blood sugar levels following a carbohydrate test meal, and (3) no loss in glycogenic function of the muscles. The evidence presented does not, we believe, aid materially in the determination of the etiology of this disease. Chronic infection may produce metabolic changes similar to those seen in these patients. But other conditions, such as avitaminosis and endocrine disturbances, may likewise produce a similar picture.

Although insulin seemed to decrease the tolerance to glucose, an improvement in appetite of all patients was noted. No definite gain in weight and no effect on the course of the arthritis were demonstrated. None of the patients studied complained of an increase in pain while on the high carbohydrate diet. There was no evidence of increased pathologic activity within the joint during this regime. The opposite was true, however, in that the patients experienced a sense of well-being and frequently asked that they be kept on a high carbohydrate diet.

SUMMARY AND CONCLUSIONS

1. The effects of a high fat diet, a high carbohydrate diet, and a high carbohydrate diet with insulin on the tolerance to dextrose was observed in three normal persons and in eleven patients with rheumatoid arthritis.

2. Simultaneous arterial and venous true sugar tolerance curves were obtained on three normal persons and on eight with arthritis. Venous curves alone were run on three persons with arthritis.

3. The tolerance to glucose was improved in all persons with arthritis and in the normal person after a high carbohydrate diet.

4. Insulin decreased the tolerance to glucose in all patients studied.

5. The evidence submitted suggests that the decreased tolerance to glucose in the individual with arthritis is due to decreased glycogenic liver function rather than to pancreatic dysfunction or circulatory stasis.

6. There was no evidence of increased pain or increased disability in the person with arthritis while on the high carbohydrate regime.

REFERENCES

1. Glover, J. A., and Newman, G.: Ministry of Health, Incidence of Rheumatic Diseases, London, No. 23, 1924.
2. United States Public Health Service: The Magnitude of the Chronic Disease Problem in the United States, National Health Survey, No. 6, 1935-36.
3. Osgood, R. B.: Human and Economic Problem of Chronic Arthritis, New England J. Med. 206: 1, 1932.

4. Hench, Philip S., Bauer, Walter, Fletcher, Almon A., Ghrist, David, Hall, Francis, and White, Preston: The Present Status of the Problem of "Rheumatism": A Review of Recent American and English Literature on "Rheumatism" and Arthritis, *Ann. Int. Med.* 8: 1315, 1935.
5. Cecil, R. L.: Rheumatoid Arthritis: A New Method of Approach to the Disease, *J. A. M. A.* 100: 1220, 1933.
6. Bauer, W., Bennett, G. A., and Short, C. C.: Speculations on the Etiology of Rheumatoid Arthritis Based Upon Physiological Studies of Normal Joints, *New England J. Med.* 208: 1035, 1933.
7. Wheeldon, T.: The Use of Colloidal Sulfur in Treatment of Arthritis, *J. Bone & Joint Surg.* 53: 879, 1935.
8. Todd, A. T.: A System of Treatment in Chronic Rheumatism, *Practitioner* 135: 692, 1935.
9. Rinehart, J. F.: Studies Relating Vitamin C Deficiency to Rheumatic Fever and Rheumatoid Arthritis; Experimental, Clinical and General Considerations, *Ann. Int. Med.* 9: 586, 671, 1935.
10. Steinberg, C. L.: Vitamin B Complex Therapy in Chronic Arthritis, *Am. J. Digest. Dis. & Nutrition* 3: 765, 1936.
11. Burbank, R.: Arthritis Considered as a Systemic Disease (Role of Streptococci), *J. M. Soc. New Jersey* 31: 522, 1934.
12. Cecil, R. L.: Medical Treatment of Chronic Arthritis, *J. A. M. A.* 103: 1583, 1934.
13. Hench, Philip S., Bauer, Walter, Fletcher, Almon A., Ghrist, David, Hall, Francis, and White, Preston: The Present Status of the Problem of Rheumatism and Arthritis, *Ann. Int. Med.* 8: 1495, 1935; *Ann. Int. Med.* 10: 754, 1936.
14. Pemberton, R.: Arthritis and Rheumatoid Conditions, Their Nature and Treatment, Philadelphia, 1930, Lea & Febiger.
15. Forbes, J. C., Neale, R. C., Hite, O. L., Armstead, O. B., and Rucker, S. L.: Studies on the Effect of a High Sulfur, Low Carbohydrate Diet in Chronic Arthritis, *J. LAB. & CLIN. MED.* 3: 1036, 1936.
16. Sweeney, J. S.: Dietary Factors That Influence Dextrose Tolerance, *Arch. Int. Med.* 40: 818-830, 1927.
17. Foster, G. I.: Sugar in Venous and Finger Blood, *J. Biol. Chem.* 55: 291, 1923.
18. Somogyi, M.: Use of Copper and Iron Salts for Deproteinization of Blood, *J. Biol. Chem.* 90: 755, 1931.
19. Shaffer, P. A., and Somogyi, M.: Copper Iodometric Reagents for Sugar Determinations, *J. Biol. Chem.* 100: 3, 1933.
20. McCulloch, E. P., and Johnson, C. R. K.: Manipulation of Glucose Tolerance by Diet, *Am. J. M. Sc.* 195: 773, 1938.
21. Corbett, J. W., and Seljeskeg, S. R.: Comparison of Sugar Tolerance Curves Obtained of Venous and Capillary Blood, *J. LAB. & CLIN. MED.* 78: 1103, 1933.

VARIATIONS IN THE COMPOSITION OF GASTRIC JUICE*

INFLUENCE OF PROTEIN ON THE ACIDITY AND PEPSIN

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THE purpose of the present series of investigations is to study systematically the effect of proteins, carbohydrates, and fats on the acidity and pepsin activity (at constant pH) of the gastric secretions.

The hitherto available data on the *simultaneous* study of the pepsin activity, pH, and free and total acidity are scarce. This is especially true for the pepsin method of Anson and Mirsky¹ and the hydrogen-ion determination by the more reliable potentiometric methods. There are no data available for studying the influence of the three main food classifications into which diets may be divided in conjunction with the sensitive double histamine test proposed by Rivers, Osterberg, and Vanzant.²

The report presented here gives the results of the protein study. The protein used was gelatin. It was selected because of its purity, availability, ease of administration, and solubility in the gastric contents.

METHODS

All observations were made on patients who gave clinical and x-ray evidence of peptic ulcer.

The procedure for carrying out the test meal was as follows: The patient presented himself in the fasting state. A Rehffuss tube was introduced into the stomach, and the fasting contents were withdrawn. Five-tenths milligram of histamine hydrochloride was then injected hypodermically, and immediately afterwards 120 c.c. of tap water were given orally. Five to ten cubic centimeter samples of gastric juice were then aspirated at fifteen-minute intervals for one hour. At the end of the hour the entire stomach contents were removed and 0.5 mg. of histamine hydrochloride was again injected. Immediately afterwards 120 c.c. of tap water were given orally. When the effect of the protein was to be determined, a suspension of 15 Gm. of U.S.P. gelatin in 120 c.c. of tap water was given. Five to ten cubic centimeter samples of gastric juice were then aspirated at successive fifteen-minute intervals for one hour. The patient was told to expectorate his saliva throughout the test.

After collection each sample was placed in the refrigerator. The samples in most instances were free from mucus and did not require filtering. The pH

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and the free and total acidity were determined within two hours after collection of the last sample. Pepsin determinations were done the following morning on a portion of the gastric juice diluted with 0.1 normal hydrochloric acid and kept in the refrigerator overnight. Pepsin values were found to be constant under these conditions.

The pH determinations were made with the Beckmann glass electrode. Free and total acidities were determined in the usual manner by titration. Values are expressed in terms of milliequivalents (meq.) per 1,000 c.c. These are numerically equivalent to what is commonly referred to as free and total acidity. The pepsin content was determined by the method of Anson and Mirsky¹ following the procedure outlined by Mullins and Flood.³ Pepsin values are expressed as milligrams of pepsin (1:4,000) per cubic centimeter of gastric juice, as suggested by Helmer, Fouts, and Zerfas,⁴ and Mullins and Flood.³ In carrying out the pepsin estimations, all dilutions were made with 0.1 normal hydrochloric acid.

Influence of Protein on the pH, Pepsin, and Free and Total Acidity of Gastric Juice During a Modified Double Histamine Test.—In testing the effect of the protein on the gastric secretions in vivo, a group of 16 patients with peptic ulcer were given the double histamine test without the protein (water group) after the first hour, and a separate group of 19 patients with peptic ulcer were given the double histamine test with the protein after the first hour (protein group).

Table I presents the mean values of pH, pepsin, and free and total acidity for these two groups. The significance of the difference between the mean values were evaluated by the statistical methods of Fisher⁵ as applied to small samples. P represents the frequency with which the difference between two means may be due to chance alone. When P is 0.05 or less, the difference between the means is considered significant.

It can be seen in Table I that during the first hour no statistically significant differences exist between the mean pH, pepsin, and free and total acidity values for the two groups. During the second hour, when one group received protein in addition to water, statistically significant differences can be noted between the mean pH, pepsin, and free acidity values for the first forty-five minutes. The total acidity values show no statistically significant changes during this period.

Thus protein in the form of gelatin when introduced into the stomach raises the pH markedly for at least thirty minutes and noticeably for forty-five minutes, markedly lowers pepsin and free acidity of the stomach for thirty minutes and noticeably for forty-five minutes, but has no effect on the total acid concentration.

Influence of the Protein on pH, Pepsin, and Free and Total Acidity in Vitro.—Since the concentration of the various constituents of the gastric juice depends on both chemical and secretory mechanisms, in vitro experiments testing the purely chemical effect of protein in the form of gelatin on acidity and pepsin were also carried out. The results are presented in Table II.

It can be seen in Table II that the protein (gelatin) markedly reduces the free acidity and causes a definite increase in the pH and a slight increase in the total acidity values. These findings are due to the acid-combining properties of

TABLE I
INFLUENCE OF PROTEIN (GELATIN) ON THE ACIDITY AND PEPSIN OF GASTRIC JUICE

	INGESTED MATERIAL	MEAN VALUES										
		TIME INTERVAL AFTER FIRST INJECTION OF HISTAMINE (MIN.)					INGESTED MATERIAL	TIME INTERVAL AFTER SECOND INJECTION OF HISTAMINE (MIN.)				
		15	30	45	60			15	30	45	60	
Free acidity*	Water	35	54	61	63		Water	50	67	69		60
P values	Water	31	49	55	58		Water + protein	11	27	42		51
Total acidity*	+	0.62	0.68	0.48	0.65			10-3	<10-5	0.02		0.11
P values	Water	48	69	77	79		Water	61	81	85		86
pH	Water	48	65	74	79		Water + protein	55	69	79		84
		1.00	0.72	0.77	1.00			0.57	0.25	0.56		0.87
P values	Water	1.97	1.41	1.32	1.30		Water	1.39	1.26	1.23		1.25
Pepsin values†	Water	1.83	1.47	1.39	1.40		Water + protein	3.25	2.57	1.97		1.69
		0.78	0.62	0.54	0.44			<10-6	10-3	0.035		0.11
P values	Water	4.9	5.7	6.1	6.6		Water	5.9	6.2	6.3		6.8
	Water	4.8	5.3	5.8	5.8		Water + protein	3.6	3.8	4.8		5.9
		0.84	0.64	0.67	0.32			<10-3	<10-3	0.05		0.25

*Expressed in milliequivalents per 1,000 c.c. of gastric juice.
†Dose, 0.1 cc. of 1% solution.

*Expressed in milliequivalents per 1,000 c.c. of gastric juice. Numerically, this corresponds to the units used clinically.
†Pepsin values are expressed as milligrams of pepsin (1:4,000) per cubic centimeter of gastric juice. P represents the frequency with which the difference between the two means may be due to chance alone.

the protein on the acid side of the isoelectric point and to the base-combining power on the alkaline side of the isoelectric point. The presence of the protein has no effect on the pepsin values, as determined by the method of Anson and Mirsky.¹

The possibility that pepsin may be destroyed when the pH of a pepsin solution is raised by addition of the protein and incubated at 37° C. for an hour was then investigated. It was found that raising the pH from 1.3 to 4.0 by the addition of the protein and thereafter incubating at 37° C. has no effect on the peptic activity, as determined by the Anson-Mirsky method.

TABLE II

INFLUENCE OF PROTEIN (GELATIN) ON THE ACIDITY AND PEPSIN IN VITRO

PROTEIN ADDED GM. PER 1,000 C.C. OF SOLUTION	FREE ACIDITY MEQ. PER 1,000 C.C.		TOTAL ACIDITY MEQ. PER 1,000 C.C.		pH			PEPSIN MG. (1:4,000) PER C.C.
0	42	91	50	100	1.0	1.3	1.6	6.3
10					1.0	1.4	1.8	6.3
20	27	76	53	103				
30								6.1
50	5	53	57	107	1.3	2.5	4.0	6.2
80					1.6	3.7	4.5	
100	0	9	64	113	2.1	4.0	4.6	6.3
120					2.9	4.2	4.7	
150	0	0	70	120	3.5	4.4	4.9	

The possibility that the pepsin digestion products of the protein would reduce peptic activity was also investigated. It was found that pepsin activity remained constant when the protein is digested at a pH of 3.0 for one hour at 37° C. This is in harmony with Northrop's experiments⁶ which show that the digestion of gelatin by pepsin takes place very slowly and then not to any great extent.

DISCUSSION

From the results obtained, it is to be seen that protein in the form of gelatin significantly reduces the hydrogen-ion concentration and pepsin, but not the total acid concentration of the gastric juice. This reduction of the hydrogen-ion concentration in the stomach is similar to the in vitro reduction of acid by the protein. The amount of reduction depends upon the quantity of acid present and the amount of protein added, as shown in Table II.

The effect of the protein on total acidity in vivo is also in harmony with the in vitro findings where (as seen in Table II) the presence of gelatin in small concentrations causes only slight variations in the total acidity. In the in vivo experiments gelatin was given in a concentration of 12 per cent. This concentration is quickly reduced by the addition of fluid from the gastric secretions, so that the concentration present in the stomach is considerably less than 12 per cent. In terms of total acidity this would mean an increase by only a few milliequivalents per 1,000 c.c. In line with this, no significant differences in the total acid was noted between the protein and control groups.

From the in vitro experiments performed, the mechanism by which the pepsin concentration is lowered in the stomach, in the presence of protein in the

form of gelatin, cannot be ascribed to any competitive retardation of peptic activity by the protein or to the destruction of pepsin when the pH is raised by the protein or to the inhibition of peptic activity by the digestion products of the protein. The factors that are possibly responsible for the lowering of the pepsin content of the gastric juice following the administration of the protein may be either a lowered volume rate of secretion of pepsin or a retardation in the rate of conversion of the pepsin precursor, pepsinogen, to pepsin.

SUMMARY

1. The influence of protein (in the form of gelatin) on the acidity and pepsin of the gastric juice of patients suffering from peptic ulcer was investigated by means of a modified double histamine test.

2. The hydrogen-ion concentration, the free acid, and the pepsin were reduced. The total titrable acidity was not appreciably influenced.

3. On the basis of in vitro experiments the reduction of the hydrogen-ion concentration and free acidity of the gastric juice may be ascribed to the acid-combining power of the protein.

4. The reduction of the pepsin content of the stomach, on the basis of in vitro experiments, cannot be ascribed to the protein per se, but possibly to a reduced secretion of pepsin or a retarded conversion of pepsinogen to pepsin.

We are indebted to Dr. C. A. Flood, of the Columbia College of Physicians and Surgeons, for his helpful suggestions regarding the technique of pepsin estimations.

REFERENCES

1. Anson, M. L., and Mirsky, A. E.: *The Estimation of Pepsin With Hemoglobin*, J. Gen. Physiol. 16: 59, 1932.
2. Rivers, A. B., Osterberg, A. C., Vanzant, F. R.: *The Double Histamine Test as an Aid in the Study of Gastric Secretory Function*, Am. J. Digest. Dis. 3: 12, 1936.
3. Mullins, C. R., and Flood, C. A.: *A Study of Gastric Pepsin in Various Diseases*, J. Clin. Investigation 14: 793, 1935.
4. Helmer, O. M., Fouts, P. J., and Zerfas, L. G.: *Gastro-Intestinal Studies. IV. The Relation of pH to the Pepsin and Rennin Content of the Gastric Juice*, Am. J. Dig. Dis. & Nutrition 1: 120, 1934.
5. Fisher, R. A.: *Statistical Methods for Research Workers*, ed. 6, London, 1936, Oliver & Boyd, p. 128.
6. Northrop, J. H.: *Comparative Hydrolysis of Gelatin by Pepsin, Trypsin, Acid and Alkali*, J. Gen. Physiol. 4: 57, 1921-22.

A CRITICAL SURVEY OF A PHENOLPHTHALEIN TEST FOR GASTROINTESTINAL LESIONS*

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IN VIEW of the need for a simple test to determine the presence of gastrointestinal lesions quickly and accurately, Woldman¹ devised a procedure based upon the urinary excretion of peroral phenolphthalein when a break exists in the mucosa of the digestive tract.

The patient in a fasting state is given 0.1 Gm. (1.5 gr.) of white phenolphthalein dissolved in 10 c.c. of 95 per cent alcohol, diluted with 20 c.c. of water. Specimens of urine are obtained two and four hours later and tested immediately for the presence of free phenolphthalein by the addition of a 10 per cent solution of sodium hydroxide. Woldman assumes that the appearance of a pink color indicates that free phenolphthalein has actually passed through a break in the continuity of the gastrointestinal mucous membrane into the blood and is finally excreted in the urine. Normally, it is excreted as conjugate phenolphthalein which fails to give this specific reaction. Prolongation of the test beyond four hours did not aid its diagnostic efficiency. Occasionally, the urinary pigments in very dark or highly colored urine presented a problem because of the masking of the faint pink color reaction of the indicator. Greene² suggested an improvement on the test by the addition of suspensions of the hydroxides of zinc, calcium, or barium to remove the pigments in the urine and at the same time produce the necessary alkalization. Comparative tests run concomitantly on such urines showed a more visible pink with these suspensions. However, it required from two to three times the amount of these alkalis to get the necessary alkalization. Therefore, whenever the test is employed, it is suggested that one of these alkalis be made available for highly colored urines.

Among the advantages of the test are enumerated its usefulness in cases where the roentgenographic diagnosis is doubtful or impossible to perform, in ruling out organic lesions of the gastrointestinal tract in diseases which may have symptoms referable to the digestive system, in differentiating functional from organic disease, and its simplicity, rapidity, and inexpensiveness. Woldman also reports an amazing accuracy for a laboratory test of its kind, with a possible error of less than 3 per cent for both positive and negative findings.

The test proved to have several serious disadvantages in our hands. The percentage of error in our series of cases is much greater than that reported, and, therefore, the test loses much of its specificity. In a series of 46 persons with proved gastrointestinal lesions, 22 failed to give a positive reaction, resulting in

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48 per cent error (Table I). A group of 105 nongastrointestinal control patients gave 25 false positive reactions, resulting in 24 per cent error (Table II). Upon repetition of the test on individuals with false positive reactions, practically the same results were obtained. The total error for both positive and negative findings was 38 per cent. These findings closely approximate the fairly uniform results obtained by other investigators (Table III).

TABLE I

RESULTS OF THE PHENOLPHTHALEIN TEST IN PATIENTS WITH GASTROINTESTINAL LESIONS

DIAGNOSIS	CASES	RESULT	
		POSITIVE	NEGATIVE
Peptic ulcer	12	7	5
Hematemesis	7	5	2
Carcinoma of stomach	9	4	5
Carcinoma of colon	4	1	3
Ulcerative colitis	5	2	3
Tuberculous enteritis	3	2	1
Amebic dysentery	1	0	1
Diverticulitis of colon	2	1	1
Acute ulcerative gastritis	1	0	1
Typhoid	2	2	0
Total	46	24	22

TABLE II

RESULTS OF THE PHENOLPHTHALEIN TEST IN PATIENTS WITHOUT GASTROINTESTINAL LESIONS

DIAGNOSIS	CASES	RESULTS	
		POSITIVE	NEGATIVE
Normal persons	10	1	9
Gall bladder disease	9	2	7
Chronic glomerulonephritis	3	1	2
Resolved pneumonias	14	4	10
Nongastrointestinal			
Malignancy (e.g., breast, lung, bone, etc.)	5	1	4
Cirrhosis of the liver	4	2	2
Cardiovascular disease (not decompensated)	25	7	18
Blood dyscrasias	5	2	3
Arthritis	7	1	6
Pulmonary tuberculosis	11	2	9
Asthma	5	1	4
Gastric neurosis	1	0	1
Upper respiratory infection	5	0	3
Diabetes	1	0	1
Total	105	26	79

In view of the fact that the tests were performed exactly as outlined by Woldman, guarding against contaminations, and testing specimens immediately following their collection, so that free phenolphthalein could not possibly be formed from the conjugated compound on standing, causes were investigated to explain these wide discrepancies. Especially was an explanation sought for the presence of the many false positive reactions. Notkin, Kirsch, and Albert⁷ believe that this is due to the fact that certain cathartics (phenolphthalein, rhubarb, senna, and cascara) impart a brown color to the urine which changes to red on the addition of alkalis. Furthermore, uroscoscin and uroerythrin produce a similar color in an alkaline medium.

TABLE III

AUTHORS	TOTAL NO. OF PATIENTS	PATIENTS WITH KNOWN GAS- TROINTESTINAL LESIONS			CONTROL PATIENTS WITHOUT GASTROINTESTINAL LESIONS		
		NO. CASES	% POSI- TIVE	% NEGA- TIVE (FALSE)	NO. CASES	% POSI- TIVE (FALSE)	% NEGA- TIVE
Kremer, D. N., Shore, P. D., and Wiesel, B. H. ¹⁹	137	23	56	44	114	21	79
Suttonfield, D. F. ²⁰	90	12	17	83	78	23	77
Notkin, L. J., Kirsch, E., and Albert, S. ³	105	13	70	30	92	37	63
Le Vine, R., and Kirsner, J. B. ²¹	115	44	62	38	71	46	54
Slutsky, B., and Wil- helmj, C. M. ²²	14	8*	36	64	6	50	50
Steigmann, F., and Dyniewicz, J. M. ⁶	200	56	78	22	144	73	27
Reich, N. E.	151	46	52	48	105	24	76

*Experimentally induced ulcers in dogs.

In an early exhaustive study Fleig⁴ proved that phenolphthalein is a stable compound which does not break down in the gastrointestinal tract into phenol and phthalic acid, and when given by mouth in larger doses, passes into the urine in its free state which may be discovered by the addition of alkali. Von Vamossy,⁵ who discovered the cathartic quality of phenolphthalein in 1902, found that more than 85 per cent of ingested phenolphthalein is eliminated in the feces of the dog and that a minimal quantity of free phenolphthalein is recovered from the urine, the remainder appearing as conjugated phenolphthalein.

Fantus and Dyniewicz⁶ indicated that after small medicinal doses free phenolphthalein is usually absent from the urine, but that the larger the dose, the more frequently does free phenolphthalein appear in the urine. The same authors,⁷ in more extensive studies on the elimination of phenolphthalein, demonstrated that three out of seven students receiving approximately the same dose (0.12 Gm.) as that used in the test, eliminated the following percentages of free phenolphthalein of the total dose: 0.1, 0.37, 1.06, respectively. Again, these authors¹⁷ recovered free phenolphthalein in 8.5 per cent of the urines of medical students and in 21.5 per cent of the urines of the patients at the Cook County Hospital without gastrointestinal lesions. Since free phenolphthalein shows its presence as a pink color in alkaline solutions in dilutions as high as 1:12,000,000 (0.1 Gm. to 120 liters),⁸ even the excretion of traces, which most investigators agree occurs, can give a positive test in normal persons, or in those without demonstrable organic gastrointestinal lesions. The occurrence of these traces of the free drug in the urine is the most important cause for its failure as a specific test in a large number of negative controls. Fantus and Dyniewicz⁶ reverse an earlier conception by concluding that the smaller the dose the larger the relative percentage of free phenolphthalein appearing, probably because the lesser amount of catharsis permits increased absorption. This may account for its urinary presence even with the smaller dosages.

More recently, Steigmann and Dyniewicz⁹ believe that there is a certain minimum of conjugated phenolphthalein below which no free phenolphthalein appears in the urine, and this amount is 5 mg. per 100 c.c. Therefore, in corroborating previous investigations,²³ the amount of conjugated phenolphthalein governs the absence or presence of free phenolphthalein. Hence, any condition which will promote an increased formation or circulation in the blood of the conjugated form will lead to the appearance of the free form in the urine. This also contradicts Woldman's assumption that free phenolphthalein depends entirely upon the presence of an ulcerative lesion. While increased intestinal absorption may play a role, the appearance of the free form depends so greatly upon the amount of the conjugated form that it is wrong, in their opinion, to attribute any special diagnostic importance to free phenolphthalein in the urine.

Patients with jaundice show practically 100 per cent free phenolphthalein present and would therefore appear to present a contraindication^{9, 10} to the test, since conjugation and excretion in the bile of the phenolphthalein do not occur in a diseased liver and it is consequently excreted in the urine in the free form.

All observers agree that cardiovascular-renal disease and the blood dyscrasias give a high percentage of positive results, probably due to greater mucosal permeability. According to Steigmann and Dyniewicz,⁹ in these cases the free phenolphthalein appears when the conjugated form is in a concentration lower than 5 mg. per cent.

It was more difficult to explain the presence of negative tests in patients with gastrointestinal lesions in which a positive test was expected. All persons tested had acute gastrointestinal complaints and all diagnoses were corroborated by laboratory and roentgenologic findings. The patients with gastrointestinal disturbances, all taken from the acute wards, suffered from peptic ulcer, gastric and colonic malignancies, ulcerative colitis, amoebic dysentery, typhoid, acute gastroenteritis, diverticulitis of the colon, intestinal tuberculosis, and hematemesis. It is possible that some of the carcinomas were not ulcerated and allowed no avenue of absorption, or that the other ulcerative lesions developed some protective antiabsorptive process. Rowland¹⁰ suggests that this is due to retained food or an adherent exudate on the ulcerations. Kremer, Shore, and Wiesel¹³ are of a similar opinion. It may be that the dosage is the important factor, since 1.5 grains of phenolphthalein may give a negative test repeatedly, while 4 grains gives a positive test regularly in the normal person.

Other objections are those associated with the gastrointestinal disturbances which the test is capable of producing (Table IV). The routine use of the alcoholic solvent (10 c.c. of 95 per cent alcohol) in cases with acute involvement of the upper tract, notably hematemesis and gastric ulcer, is considered inadvisable. It has a tendency to produce temporary gastric distress and enhances the possibility of further bleeding. Clark¹¹ commented on the fact that alcohol taken upon an empty stomach in concentrations above 10 per cent produces gastric irritation and causes an initial secretion of gastric juice which is followed by an inhibition persisting for as long as twenty-four hours. The resulting increased gastric secretion is, of course, especially inadvisable in peptic ulcer. Other vehicles for the phenolphthalein were found by Fantus and

Dyniewicz,⁶ after a comprehensive study of solvents, to be unsuitable because of insolubility or marked irritability, making the use of an alcoholic solvent imperative.

TABLE IV
TOXIC EFFECTS OF THE PHENOLPHTHALEIN TEST

	CASES	PERCENTAGE
Nausea	41	30.0
Vomiting	9	6.5
Abdominal cramps	16	11.7
Diarrhea	11	8.0
Laxative effect	72	47.0
Anorexia	20	13.2
Skin	0	0
Pyrexia	5	3.6
Kidney manifestations	0	0

Many of our patients complained of nausea and a few vomited; the vomiting may have been due in part to a superimposed psychic factor. Indigestion of subsequent food or anorexia was a source of annoyance for another group of patients. The most serious digestive derangement, however, was diarrhea. Although Woldman states that a small dose of 0.1 Gm. is without laxative effect, Fantus and Dyniewicz⁷ record these effects in practically all of their normal individuals, even with approximately half as large a dose as that employed in the test (0.06 Gm.). Some degree of laxation occurred in almost half our patients, while others⁹ report it in as high as 80 per cent of their patients. Abramowitz⁸ has indicated that the laxative action of phenolphthalein is further enhanced when the drug is ingested in an alcoholic solution. Gunn¹² pointed out that after its solution by bile and alkali in the intestine, phenolphthalein produces purgation by mild irritation of the small bowel and especially the large bowel. Some of it may be absorbed and re-excreted into the intestine so that its purgative action may last for a few days. Such was the case in our group of patients, especially those with typhoid, ulcerative colitis, or gastric malignancy. It was even more pronounced where diarrhea existed previously. Loose movements persisted anywhere from one to three days and were accompanied by a sudden rise in temperature, ranging between 101° and 104° F., notably with lesions of the lower bowel. Abdominal cramps lasting several hours also occurred, especially if diarrhea was present. This was undoubtedly due to the irritant effect of both the solvent and solute as previously described. Newman,¹³ in enumerating seventeen types of atypical skin lesions, visceral hemorrhages and ulcerative colitis, as well as other evidences of phenolphthalein toxicity, mentioned that even small amounts used for "pink icing on cakes, for coloring of candies, and in pink mouth washes and dentifrices" may cause these lesions.

We did not notice any skin manifestations in any of the cases, although such lesions of the fixed erythematous and multiforme types have been described by Fox,¹⁴ Wise and Abramowitz,¹⁵ Newman,¹³ and others. Eruptions due to phenolphthalein, although not very frequent, should be watched for. No evidences of renal irritation or the constant slight albuminuria after small doses of 0.05 to 0.15 Gm., described by Hydrick,¹⁶ were noted. However, this has not been confirmed by Abramowitz,⁸ Fantus and Dyniewicz,¹⁷ and others.

In a comprehensive survey of all phenolphthalein reactions reported up to 1935, Abramowitz⁸ stated the advisability of first testing a patient for susceptibility to phenolphthalein with a small dose of 0.032 Gm. (0.5 gr.) or even less. The type of idiosyncrasy that exists points toward a sensitization of the patient to the drug, especially in view of the small dosage and the occasionally long intervals between ingestion of the drug and the reactions.

Although von Vamossy¹⁸ raised objections against the existence of numerous reactions which have been accumulated to date, the evidence remains overwhelmingly in favor of their occurrence (except possibly for the presence of albuminuria), making the test further objectionable in numerous cases.

CONCLUSIONS

1. The phenolphthalein test for gastrointestinal lesions, although simple and rapid to perform, is not without many important disadvantages. The error in our series is 24 per cent for controls and 48 per cent for persons with definite gastrointestinal lesions. Other investigations concur in the high percentage of error.

2. Explanations are offered for the marked discrepancies occurring in both false positive and false negative reactions.

3. The test is definitely contraindicated in hematemesis, most cases of acute ulceration, and acute diseases of the colon or acute exacerbations of chronic ulcerative conditions. It is also valueless in cases of jaundice, cardiovascular-renal disease, and blood dyscrasias.

4. Among the toxic gastrointestinal disturbances resulting are nausea, vomiting, laxation, abdominal cramps, diarrhea, temperature elevation and, more rarely, skin manifestations.

5. The phenolphthalein test cannot be recommended as a diagnostic procedure for the differentiation of organic disease from functional disorders of the gastrointestinal tract, or between gastrointestinal disease and diseases outside the digestive system which display symptoms referable to the stomach or intestines.

REFERENCES

1. Woldman, E. E.: A Simple Test for Determining the Presence of Gastro-Intestinal Lesions, *Am. J. Digest. Dis. & Nutrition* 5: 221, 1938.
2. Greene, C. H.: Personal communication.
3. Notkin, L. J., Kirsch, E., and Albert, S.: Note on the Value of Woldman's Phenolphthalein Test for Gastro-Intestinal Lesions, *Am. J. Digest. Dis.* 6: 365, 1939.
4. Fleig, M. C.: Deux Purgatifs Synthétiques, *Arch. Int. Med.* 18: 327, 1908.
5. von Vamossy, Z.: Ueber ein Neues Abfuhrmittel (Purgen), *Therapie der Gegenwart*, München med. Wchnschr., May, 1902 (Quoted by Fantus and Dyniewicz).
6. Fantus, B., and Dyniewicz, J. M.: IV. Phenolphthalein Solubilities, *Am. J. Digest. Dis. & Nutrition* 3: 573, 1936.
7. Fantus, B., and Dyniewicz, J. M.: Phenolphthalein Studies: Elimination of Phenolphthalein, *J. A. M. A.* 110: 796, 1938.
8. Abramowitz, E. W.: Reactions Due to Phenolphthalein, *Arch. Dermat. & Syph.* 31: 777, 1935.
9. Steigmann, F., and Dyniewicz, J. M.: Urinary Elimination of Free Phenolphthalein: No Test for Gastro-Intestinal Ulceration, *Am. J. Digest. Dis.* 6: 120, 1939.
10. Rowland, V. C.: Editorial: The Controversy Over the Woldman Phenolphthalein Test for Gastro-Intestinal Ulceration, *Am. J. Digest. Dis.* 6: 396, 1939.
11. Clark, A. J.: *Applied Pharmacology*, ed. 5, Philadelphia, 1932, P. Blakiston's Son & Co., p. 287.
12. Gunn, J. A.: *An Introduction to Pharmacology and Therapeutics*, ed. 2, London, 1931, Oxford University Press, p. 44.

13. Newman, Ben A.: Phenolphthalein Intoxication, *J. A. M. A.* 101: 761, 1933.
14. Fox, H.: Erythema Perstans Following Ingestion of Phenolphthalein, *J. Cutan. Dis.* 36: 252, 1918.
15. Wise, F., and Abramowitz, E. W.: Phenolphthalein Eruptions, *Arch. Dermat. & Syph.* 5: 297, 1922.
16. Hydrick, J. L.: Albuminuria Following Ingestion of Phenolphthalein, *J. Biol. Chem.* 17: 36, 1914.
17. Fantus, B., and Dyniewicz, J. M.: Phenolphthalein Studies: 1,000 Doses of Phenolphthalein: Urinalyses, *J. A. M. A.* 108: 439, 1937.
18. von Vamossy, Z.: Is Phenolphthalein Harmful? *Am. J. Digest. Dis. & Nutrition* 3: 22, 1936.
19. Kremer, D. N., Shore, P. D., and Wiesel, B. H.: Phenolphthalein as a Test in Gastro-Intestinal Disease, *Am. J. Digest. Dis.* 6: 192, 1939.
20. Suttentfield, D. F.: The Proposed Test for Determining the Presence of Gastro-Intestinal Lesions, Results in 94 Cases, *Am. J. Digest. Dis.* 6: 206, 1939.
21. Le Vine, R., and Kirsner, J. B.: An Evaluation of the Phenolphthalein Test of Woldman, *Am. J. M. Sc.* 198: 389, 1939.
22. Slutsky, B., and Wilhelmj, C. M.: Phenolphthalein as a Test for Gastro-Intestinal Ulceration in the Experimental Animal, *Am. J. Digest. Dis.* 6: 449, 1939.
23. Abel, J. J., and Rowntree, L. G.: Action of Some Phthaleins and Their Derivatives, *J. Pharmacol. & Exper. Therap.* 1: 231, 1909.

75 OCEAN AVENUE
BROOKLYN, N. Y.

LABORATORY METHODS

GENERAL

AN INEXPENSIVE POLARIZING DEVICE FOR MICROSCOPES*

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THE study of urinary sediments of patients with renal disease, especially when massive albuminuria is present, should always include the use of a polarizing microscope to discover the presence of doubly refractile bodies (anisotropic lipoids). Nicol prisms serve excellently to convert a standard into a polarizing microscope at a cost of about \$50.00. Recently, polaroid material mounted between white optical glass was introduced as a less expensive substitute for Nicol prisms. The price of this polarizing equipment, from \$15.00 to \$30.00, may be too costly for many physicians for such specialized microscope accessories. During the past two years we have utilized the relatively inexpensive polaroid film of 3/1,000 inch thickness to convert a standard into a polarizing microscope at a cost of \$2.00. In order to enable physicians to study urinary sediments in their own laboratories by polarized light with this inexpensive medium, we are presenting our method of converting the standard laboratory microscope into one which will serve this purpose.

Polaroid film may be purchased† in four inch squares at a cost of \$4.00 each (sufficient for two microscopes). Care must be exercised in handling the film to avoid surface scratches. The eyepiece is removed from the microscope, and its outer lens mount is unscrewed. On the outer circular rim on the under-surface of the lens mount, a thin layer of clear, neutral Canada balsam is carefully applied. A circular piece of polaroid film is cut from the four inch square of a diameter just a trifle smaller than the total diameter of the rim, and is carefully applied to the rim over the balsam. After the balsam has hardened, within forty-eight to seventy-two hours, the outer lens mount is screwed into the eyepiece. The latter is replaced into the microscope and will serve as the analyzer. A wider circular piece of film is then carefully cut from the remainder of the four inch square to fit the horizontal slot under the condenser below the stage of the microscope. This larger piece of film is carefully inserted into the slot; it should not be removed thereafter. It will serve as the polarizer.

The converted microscope may be used for the usual laboratory purposes by rotating the eyepiece so that the axes of the analyzer and polarizer are

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†Polaroid film may be purchased from the Polaroid Company, 285 Columbus Avenue, Boston.

parallel. By slow rotation of the eyepiece one produces a reddish-purplish-black field (when the analyzer and the polarizer are at right angles) in which doubly refractile bodies in the urinary sediment will appear as glistening maltese crosses. Care must be exercised to distinguish these bodies from debris, crystals, etc.

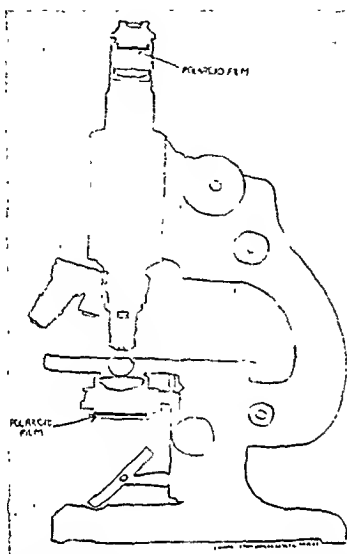


Fig. 1.—Diagram of polarizing microscope showing location of polaroid film.

With the polarizing microscope, prepared according to the method described (Fig. 1), we have found doubly refractile bodies in the urinary sediments of patients with the nephrotic syndrome associated with glomerulonephritis, amyloid disease, thrombosis of the renal veins, diabetes and hypertension.

THE MEASUREMENT OF RED BLOOD CELL DIAMETER BY THE DIFFRACTOMETER*

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THE measurement of red blood cells by the diffraction or halo method was first described by Pijper¹ in 1924. He seems to have used it only in the diagnosis of pernicious anemia, as did Eve.² Because it was of simple construction and required little time it was soon to come into wider clinical use.³⁻⁵ However, there is little report of a halo method gaining popularity in this country, and only two of our textbooks^{6, 7} consider the procedure in detail.

In view of the fact that so little actual experience has been reported, we would like to present our impressions. Since July, 1936, we have been using a diffractometer manufactured by the Spenceer Lens Co. The instrument itself is very easy to use and may be attached to any monocular microscope. There is no optical mechanism involved. A small black cylinder with a pin hole in the bottom is attached in place of the objective. The eyepiece is replaced by a second cylinder with a pinhole in the top and an adjustable device on the sides for measuring the halo. The microscope mirror is adjusted to give the most light from any good lamp (see Fig. 1). The slide is inserted in a slot in the top cylinder and rests above the measurer. Either a stained or an unstained smear may be used, but the blood side should be placed down.

When one looks into the instrument he sees a white spot in the center surrounded by a yellow halo which shades into a red periphery. The two parallel black bands on the outside are adjusted so that they just touch the outer rim of the red halo. The reading is then taken from the sliding scale and compared with a conversion table to give the mean red blood cell diameter in microns.

We have done approximately a thousand determinations by this method. Every patient having even a mild degree of anemia has at least one mean cell diameter, more often he has several. Very high white counts interfere with a distinct halo; for this reason, we do not attempt readings in patients with leucemia. Occasionally polycythemia offers technical difficulties and an extreme degree of anisocytosis may blur the outline of the halo.

The success of securing an accurate reading lies in having a smear of one cell thickness. The good smears give a clear-cut halo, and it is easy to determine the exact edge of the red circle. Herein lies one of the main sources of error, for two observers may not agree on the outline and thus may obtain different readings. Table I shows how these results may vary between two people. The maximum variation between the readings of two observers using the same smear is 0.4μ , as compared with 0.3μ when the same observer used different slides. Even this slight error makes very little diagnostic difference.

*From the Presbyterian Hospital, New York.
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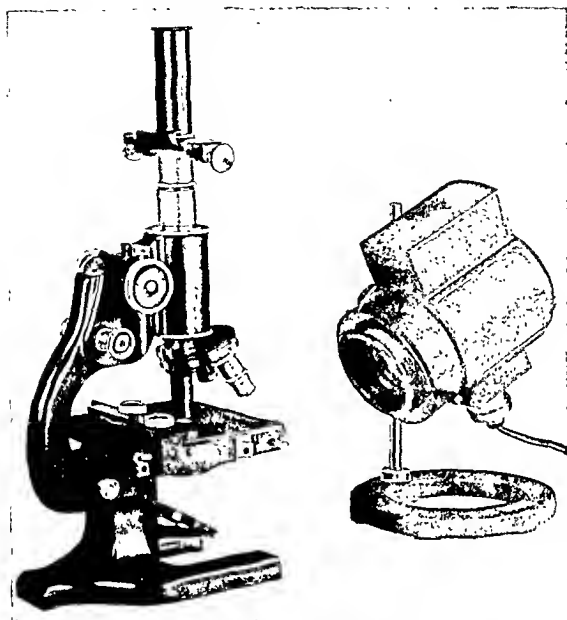


Fig. 1.—The diffractometer. (Courtesy of Spencer Lens Co.)

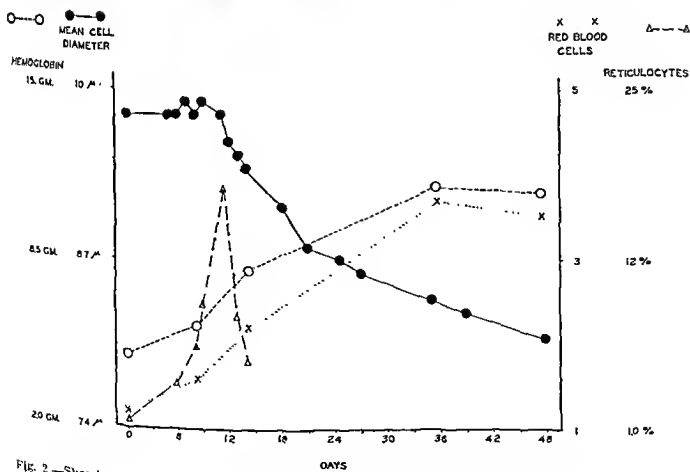


Fig. 2.—Showing the mean cell diameter, hemoglobin, red blood cell, and reticulocyte response to liver extract in a patient with pernicious anemia.

TABLE I

SHOWING VARIATIONS IN MEAN CELL DIAMETER READINGS ON TWO PATIENTS AS OBTAINED BY TWO OBSERVERS USING THE SAME SLIDE AND DIFFERENT SLIDES

PATIENT M. M.				PATIENT A. C.			
DATE	SLIDE	1ST OBSERVER	2ND OBSERVER	DATE	SLIDE	1ST OBSERVER	2ND OBSERVER
2/17/39	1	9.6	9.6	3/23/39	1	8.4	8.5
2/18/39	1	8.8	9.2		2	8.4	8.4
	2	9.0	9.3	3/24/39	1	8.6	8.4
	3	8.8	9.0		2	8.4	8.4
	4	8.9	9.1	3/25/39	1	8.3	8.4
2/20/39	1	9.1	9.3		2	8.4	8.4
	2	9.2	9.4	3/28/39	1	8.3	8.5
2/21/39	1	8.9	9.1		2	8.4	8.5
	2	9.1	9.0	3/29/39	1	8.3	8.4
2/23/39	1	9.2	8.9		2	8.5	8.5
	2	9.3	9.0	3/30/39	1	8.7	8.5
2/28/39	1	8.9	8.8		2	8.6	8.5
	2	9.1	8.9	3/31/39	1	8.6	8.4
3/ 3/39	1	8.9	8.9		2	8.7	8.4
	2	8.9	8.9	4/ 3/39	1	8.4	8.5
3/ 4/39	1	8.8	8.9		2	8.5	8.4
	2	8.8	8.8	4/ 4/39	1	8.5	8.5
3/ 7/39	1	8.7	8.9		2	8.5	8.5
	2	8.7	8.7	4/ 5/39	1	8.5	8.4
3/ 8/39	1	9.9	8.8		2	8.4	8.5
	2	9.0	8.8	4/ 6/39	1	8.6	8.5
3/ 9/39	1	8.9	8.8		2	8.4	8.5
	2	8.8	8.8	4/ 7/39	1	8.4	8.4
3/13/39	1	8.9	9.0		2	8.5	8.3
	2	8.8	8.8				

TABLE II

MEAN RED BLOOD CELL DIAMETER RESULTS OBTAINED BY THE DIFFRACTOMETER METHOD COMPARED WITH THE PRICE-JONES TECHNIQUE

PATIENT	PRICE-JONES METHOD		DIFFRACTOMETER METHOD	
	1ST OBSERVER	2ND OBSERVER	1ST OBSERVER	2ND OBSERVER
1	7.0	7.8	6.9	7.0
2	7.5	7.9	7.6	7.7
3	7.2	8.1	7.2	7.3
4	7.5	7.8	7.5	7.5
5	7.3	7.9	7.2	7.2
6	7.7	8.1	7.5	7.6
7	8.5	9.0	8.9	9.1
8	7.7	8.5	7.9	7.7
9	6.9	8.0	7.6	7.4
10	7.8	8.7	7.5	7.5

As a matter of interest two of us obtained mean cell diameters on the same smears by the Price-Jones and Diffractometer methods. Table II shows a difference of as much as 1.1μ with the Price-Jones method, and only 0.2μ with the diffractometer method. Needless to say, there is a vast difference in the time consumed by the two procedures, the halo method requiring only a minute or two, and the direct measuring thirty minutes or more.

The halo method may be criticized because it does not measure cell thickness. However, there are few cases of anemia in which mean corpuscular volume is more helpful in determining microcytosis or macrocytosis than mean cell diam-

eter. Technical errors may limit the accuracy of both methods proportionately. A smear preparation is much easier to obtain from the standpoint of both the patient and technician.

No claim of perfection is made for the diffractometer, but over a period of three years it has proved itself a simple means of obtaining red blood cell size within a reasonable range of error. It is an instrument which might be a real help in any hematologic laboratory.

CONCLUSIONS

1. In over 1,000 mean cell diameter determinations the diffractometer has been found to be simple, efficient, and sufficiently accurate for all clinical purposes.

2. Tables are presented to show that the errors inherent in the method are relatively insignificant.

3. The more general use of this method is suggested.

I am indebted to Miss Illyne for her technical assistance.

REFERENCES

1. Pijper, A.: Diagnosis of Addison's (Pernicious) Anaemia by a New Optical Method, *Lancet* 2: 367, 1924.
2. Eve, F. C.: Early Diagnosis of Pernicious Anaemia by Halometer, *Brit. M. J.* 2: 48, 1929.
3. Pijper, A.: Improved Diffraction Method for Diagnosis and Following Course of Pernicious and Other Anaemias, *Brit. M. J.* 1: 635, 1929.
4. Emmons, W. F.: Clinical Eriometer, *Quart. J. Med.* 21: 83, 1927.
5. Pryce, D. M.: Simplification of "Halo" Method of Measuring Diameter of Red Blood Corpuscles, *Lancet* 2: 275, 1929.
6. Nicholson, Daniel: *Laboratory Medicine*, Philadelphia, 1934, Lea & Febiger, p. 152.
7. Osgood, Edwin E.: *Laboratory Diagnosis*, Philadelphia, 1940, P. Blakiston's Son & Co., p. 495.

AN ECONOMICAL AND EFFICIENT DISTILLING DUST TRAP

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PRACTICALLY no laboratory worker has failed at one time or another to experience the need for an efficient means of keeping dust from entering the distillate in routine laboratory distillations.

For a number of months I have used one of the ordinary spiral waxed paper drinking cups, such as is usually found in almost every hospital or business institution. These cups are conical in shape, and in use the tip is simply cut off and the cup pushed up on the condenser tube with the large end down. In this manner the cup may be slipped down so that it covers the mouth of the receiving flask, thus providing a protective shield against dust.

A CONTRAST STAINING METHOD FOR HEMOSIDERIN PIGMENT IN HEART-FAILURE CELLS

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THE methods in use to demonstrate hemosiderin pigment offer no means of differentiating the heart-failure cells from the surrounding leucocytes. In some cases it is difficult to demonstrate these cells because no contrast staining is offered. Isolation of such cells is also difficult because the entire smear is colorless save for the stained hemosiderin pigment granules which themselves appear in unstained cells.

A new staining method here presented allows for a contrast. In the sputum or urine, or other material for examination, leucocytes appearing with heart-failure cells are counterstained by Wright's stain, and the hemosiderin takes a bright green stain. The hemosiderin pigment stands out in sharply contrasted granules against the well-stained basic blue and acidophilic red of the stained leucocytes and other cells.

Method for Staining.—(1) Make smear of sputum in usual way. (2) Air dry. (3) Fix with heat. (4) Apply a 10 per cent solution of potassium ferrocyanide and an equal quantity of tenth-normal hydrochloric acid to the slide. Flood the slide thoroughly. (5) Allow this to stand for fifteen minutes. (6) Wash in water. (7) Dry thoroughly. (8) Apply Wright's stain. (9) Add buffered water for five minutes. (10) Wash in water. (11) Dry thoroughly and examine under oil.

A NEW MODEL MOUSE CAGE

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A NEW type of cage provides optimum living conditions for animals, which are indispensable requisites for trustworthy experimental work in biological laboratories. Fig. 1 shows the cage and run-way ready for use except nesting material. Fig. 2 shows the nest and run-way disconnected. The length over-all is 36 inches; the run-way is 30 by 4 by 3 inches.

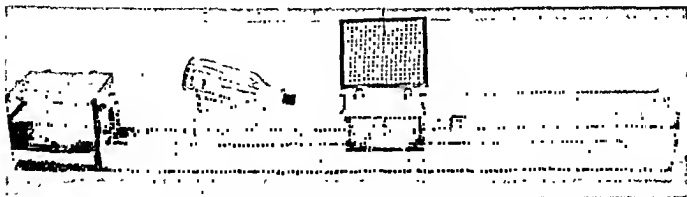


Fig. 1.—A, False bottom for nesting cage; B, nesting cage, $5\frac{1}{4}$ by $5\frac{1}{4}$ by 4 inches, holds 5 or 6 adult mice; C, lid giving access to run-way; D, false bottom—aluminum 30 by $3\frac{3}{4}$ inches, $\frac{1}{4}$ inch curb; E, wire fastener and door—aluminum—for closing end of run-way; F, salad holder which prevents contamination of fresh vegetables and fruits.

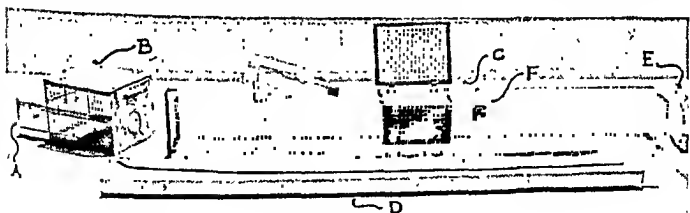


FIG. 2.

The black space on the side of the run-way is the feed box. The water bottle is supported by a wire eradle. The nest is supported securely and easily on the run-way by a metal strip pointing downward toward the circular opening, which engages with a vertical strip on the top of the run-way. Removal and replacement of the nest are thus made easy.

A thick mat of paper pulp—heavy blotting paper—covers the false bottom of the run-way. It is to be replaced by a fresh one as soon as it becomes soiled. This is accomplished quickly and easily by opening E, withdrawing the soiled mat and inserting a clean one. The mice prudently deposit their excreta at E, as far away from the nest as possible.

To economize laboratory floor space, the cages are supported on a rack which holds sixteen units. Forty-eight cages on three racks, housing 250 mice, would require only a small corner of a room, 4 by 11 feet.

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The square boxcage, shown on the floor of Fig. 3, is a standard type of cage used extensively in biological laboratories. Ventilation, sanitation and opportunity for exercise are utterly inadequate. The long run-way of the new



FIG. 3.

model gives an abundance of fresh air and unlimited chance for exercise. Note the mouse walking upside down on the ceiling of the fifth cage on the rack. The hygienic advantages of the modern cage over the old standard type are glaringly obvious. This kind of cage is admirably adapted for high school biology classes on account of the minimum amount of time required to keep the unit tidy and odorless, simply by replacement of the floor mat once daily.

A SIMPLE AND ACCURATE MICROFRAGILITY TEST FOR MEASURING ERYTHROCYTE RESISTANCE*

A COMBINATION MICROHEMOPIPETTE METHOD

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NUMEROUS methods have been devised and revised for the purpose of testing the resistance of the red blood cells to various hemolytic and hemotoxic agents. Quantitation of hemolysis may be expressed in terms of any of the following criteria: (1) the depth of red color due to dissolved hemoglobin developing in the hemolytic media in which the erythrocytes have been suspended; (2) the percentage volume of the unhemolyzed corpuscles, as determined by the hematocrit method after centrifugation, since the hypotonic solutions cause the cells to expand until the maximum distensibility (the "critical" volume) has been obtained; or (3) the number of intact erythrocytes remaining in the hemolytic solutions, as enumerated by the hemocytometer method. These criteria may be used singly or in combination.

Each of the above-mentioned methods of measurement possesses its own merit and yields satisfactory results in the hands of experienced observers who have mastered its technical details. The simplest and perhaps the most reliable criterion, however, appears to be the one which estimates the degree of hemolysis by the tintorial density of the red supernatant fluid resulting from the presence of varying amounts of hemoglobin liberated from ruptured corpuscles. This is the oldest principle of the test as first devised by Hamburger (1883) and adapted for clinical use by von Limbeck (1896) and by Ribierre (1903). Because of its simplicity and accuracy, this method, with repeated modifications by Theobald Smith (1904), Karsner and Pearce (1912), Hill (1915), Giffin and Sanford (1919), Fontaine (1921), Daland and Worthley (1935), and Beebe and Hanley (1936), is still today most extensively used.

The hematocrit method, first thoroughly investigated by Hamburger (1902) and simplified by van Allen (1925) and his followers, particularly Silvette (1927) and Guest and Wing (1939), is unsatisfactory unless accompanied by other criteria, since the estimation of packed cell volume often yields doubtful results, primarily because of the presence of "ghost" cells and of unavoidable errors arising from differences in the speed and duration of centrifugation. This is particularly true when determinations are to be made on patients with severe anemia accompanied by oligocythemia.

Enumeration of intact erythrocytes remaining in the hypotonic solutions of varying concentrations is also an old method, first proposed by Chanel

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(1880) and later refined by Simmel (1923), Leake and Pratt (1925), Waugh and Chase (1928), and Whitty and Hynes (1935). This method may be accurate, but the presence of hemolyzed cells with intact stroma among the truly unhemolyzed cells may lead to confusing results. Moreover, the use of a counting chamber for each concentration of salt solutions is time-consuming, and the preparation of hypotonic solutions, as devised by Simmel, is complicated. To overcome this defect, Harvey (1937) proposed the use of only one pipette and a standard hypotonic diluent of 0.35 per cent solution of sodium chloride.

A variety of hemolytic and hemotoxic substances have been employed to induce hemolysis for testing the erythrocyte resistance. The agent longest in use is sodium chloride in different concentrations, this having been first utilized by Johannes Duncan in 1867 and by Malassez in 1872, and in 1900 Vaquez pronounced a scale consisting of gradually increasing dilutions to be the "ideal method." Other substances used for the purpose are sodium oxalate, sodium sulfate, saponin, and sapotoxin, as well as other vegetable and animal poisons. The stock solution recommended by Simmel contains sodium chloride, potassium chloride, magnesium chloride, calcium chloride, sodium biphosphate, and sodium bicarbonate. Another method, employed by Theobald Smith (1904), and by Wiseman and Bierbaum (1932) is that of using diluted serum or plasma obtained from the blood samples to be tested for erythrocyte fragility. The Grancher's serum used by Chancel contained one gram of sodium sulfate in 40 c.c. of serum.

The hemolytic action of these various substances upon erythrocytes differs according to physical and chemical characteristics. Certain chemicals (benzene and indol) cause the cell membrane to become more permeable, while others (sugar and lecithin) increase the resistance of erythrocyte stroma. The presence in the blood serum of inhibitory or accelerative substances is also an important factor in understanding the mechanism of hemolysis in vitro as well as in vivo.

Nearly all the methods mentioned, except the hematocrit method, require the use of venous blood, a feature which offers a decided obstacle in testing the fragility of red blood cells in small infants. The method herein described is an attempt to reduce the technique of erythrocyte fragility test to its simplest terms by using only six different concentrations of hypotonic sodium chloride solutions and the least possible amount of capillary blood. Incidentally, it must be emphasized that the micromethod here described, because of the exactness required in mixing the saline with distilled water in correct proportions, yields results that are far more accurate than any of the prevailing macroscopic methods.

DESCRIPTION OF THE MICROFRAGILITY TEST

Instruments and Utensils Required for the Test.—For the performance of the microfragility test a set of six combination microhemopipettes* with metal holders (Kato, 1938) are used. The hypotonic solutions are prepared from 0.9 per cent solution of chemically pure recently dried sodium chloride in distilled water, the dilutions being made according to the proportions recommended

*Combination microhemopipettes and metal holders are manufactured by the Fisher Scientific Co., Pittsburgh, Pa.

in Table I. For obtaining the blood sample (see below) a hanging-drop slide with a central well (14 mm. in diameter and 3 mm. in depth) is employed.* For mixing the blood with hypotonic solutions a white porcelain spot plate provided with twelve depressions is most useful, since the entire series of dilutions can be prepared in rapid succession.

TABLE I

GRADUATED SCALE OF CONCENTRATIONS OF SODIUM CHLORIDE SOLUTION
RECOMMENDED FOR USE IN MICROFRAGILITY TEST

PIPETTE NO.	NaCl (0.9%) (c.mm.)	DISTILLED WATER (c.mm.)	OXALATED BLOOD (c.mm.)	SALT CONC. (c) (%)	NORMAL HEMOLYSIS SPAN
	30	18	12	0.630	Goat
	29	19	12	0.615	
	28	20	12	0.600	
	27	21	12	0.585	
	26	22	12	0.570	
	25	23	12	0.555	Rabbit
	24	24	12	0.540	
	23	25	12	0.525	
	22	26	12	0.510	
	21	27	12	0.495	
	20	28	12	0.480	Man
	19	29	12	0.465	
1	18*	30	12	0.450	
2	17*	31	12	0.435	
3	16*	32	12	0.420	
4	15*	33	12	0.405	Man
	14	34	12	0.390	
	13	35	12	0.375	
5	12*	36	12	0.360	
	11	37	12	0.345	
6	10*	38	12	0.330	
	9	39	12	0.315	
	8	40	12	0.300	
	7	41	12	0.285	
	6	42	12	0.270	
	5	43	12	0.255	

*Indicates the most important concentrations which must be included in the test.

The total amount of hypotonic salt solution in all the concentrations tabulated above is 60 c.mm., allowing 10 c.mm. for loss incident to manipulation.

The salt concentration (C) of any mixture may be calculated from the equation:

$$\frac{0.9\% \text{ NaCl (c.mm.)} + \text{Blood (c.mm.)}}{60} \times 0.9 = C$$

Obtaining Blood Samples.—Capillary blood is obtained from a deep skin puncture in the usual manner and is brought immediately into contact with either dry heparin powder or double oxalate mixture, the latter of which will enable the observer to use the same sample for testing the prothrombin clotting time if indicated (Kato, 1940). The proportion of either anticoagulant to the amount of blood sample should be in the neighborhood of 1:500. In this proportion these anticoagulants exert a negligible influence upon the osmotic equilibrium of the blood cells and produce no alterations on the packed cell volume. Thus, for the performance of microfragility test approximately 0.2 c.c. of capillary blood is received into the well of a hanging-drop slide, containing 0.4 mg.

x. y. *Hanging-drop slides (culture slides) can be obtained from Clay-Adams Co., New York.

of an anticoagulant. This amount of anticoagulant can be accurately delivered by introducing 20 c.mm. of a 2 per cent solution. The double oxalate solution has the following composition:

Potassium oxalate	0.75 Gm.
Ammonium oxalate	1.25 Gm.
Distilled water	100.00 c.c.

The heparin solution of similar concentration is made from crude heparin in distilled water.

The blood sample prepared according to the foregoing method is then placed in a moist chamber until the test is performed.

Preparation of Erythrocyte Suspensions.—To prepare the suspensions of erythrocytes in hypotonic solutions, the specified amount of 0.9 per cent stock solution of sodium chloride is first drawn up into a combination microhemopipette and carefully expelled into one of the depressions of a spot plate. Using the same pipette, the distilled water, also in the corresponding specified amount, is measured out and added to the saline. The two reagents are then gently but thoroughly mixed by alternately drawing up and expelling the mixture in the bore of the pipette. Finally, with the same pipette the specified amount of well-homogenized blood is thoroughly mixed with the hypotonic solution, and the resulting suspension is at once drawn up to the top mark (50 c.mm.) of the pipette, thus avoiding any waste of the suspension. The total amount of suspension, prepared according to the proportions recommended in Table I, is exactly 60 c.mm., allowing for loss of approximately 10 c.mm., which usually adhere to the surface of the utensil during the preparation of the mixture. This manipulation requires a little skill which, however, can be mastered easily after a few trials. If the blowing force is too strong, bubble formation in the mixture will invariably cause a shortage of the suspension to fill the pipette to the top mark. If too much of the suspension is lost in this way, or in any other way, a new one must be prepared to insure uniform results, especially with reference to the cell volume in each pipette.

After the suspension is drawn up to the top mark, the two ends of the pipette are at once closed by immobilization in the metal holder, and correctly labeled according to the number corresponding to each dilution. The same process is repeated for each concentration of salt solutions. After the entire series of dilutions have been prepared, they are left in horizontal positions on a flat surface and allowed to remain so for at least one hour, occasionally reversing the positions of the pipettes to permit the complete hemolytic action of solutions upon the suspended erythrocytes. The pipettes in their metal holders are then centrifuged for ten to fifteen minutes at 2,500 r.p.m. The supernatant fluid in each pipette and the cellular layer at its bottom may now be observed as to the extent of hemolysis.

Recording the Results.—The concentrations recommended for the series of six pipettes used in the microfragility test have been selected to include only the two critical ranges; namely, the range in which initial hemolysis normally occurs (first four pipettes) and the range in which complete hemolysis is most frequently observed (last two pipettes). For normal human blood beyond in-

fancy the concentration at which initial hemolysis occurs is usually 0.435 per cent (the second pipette), and occasionally 0.420 per cent (the third pipette), and complete hemolysis is 0.330 per cent concentration (the last pipette). These two critical concentrations can be used to designate the span of hemolysis, conveniently recorded as 0.435/0.330. Undoubtedly, even under normal conditions slight variations may occur, but the selection of the two critical ranges recommended is based upon experience gained in performing a large number of tests on normal human blood. If too great a deviation is found in either direction, a repetition of the test is necessary by changing the scale of concentrations in whichever direction indicated.

TABLE II
AVERAGE NORMAL FRAGILITY OF HUMAN ERYTHROCYTES
(Microfragility Test)

PIPETTE NO.	SALT CONC. (%)	DEGREE OF HEMOLYSIS	VOLUME OF GHOST CELLS (C.MM.)	VOLUME OF INTACT CELLS* (C.MM.)
1	0.450	0	0	5.0
2	0.435	±	0.1	5.5
3	0.420	+	0.5	6.0
4	0.405	++	1.0	5.0
5	0.345	+++	Indistinct	2.0
6	0.330	++++	Invisible	0

*The figures in this column represent the values of packed cell volume of unhemolyzed erythrocytes in actual number of cubic millimeters after centrifugation (compare with Fig. 1). Since these values depend upon the percentage volume of packed erythrocytes in undiluted whole blood, the volume in each diluted specimen may be more accurately expressed in relative percentage of the packed whole cell volume.

In recording the degree of hemolysis the depth of the red color, due to the presence of dissolved hemoglobin, affords the most reliable criterion, but the packed erythrocyte column found at the bottom of the pipette also yields valuable information (Table II). In the second pipette, in which initial hemolysis normally occurs, the red color of the supernatant fluid may be so slight that its detection may require a careful comparison with the first pipette in which hemolysis is negative. But a trace of hemolysis in the second pipette is almost invariably accompanied by the presence of a very thin layer of transparent hemolyzed cells, the so-called "ghost" cells, overlying the packed intact erythrocytes. As the concentration of the salt solution decreases, the "ghost" cell layer will be seen to increase in proportion to the augmenting intensity of the red color in the supernatant fluid. At the same time there is a gradual decrease in volume of opaque intact erythrocytes after having increased at initial hemolysis, until complete hemolysis is reached, at which point there is no demonstrable column of intact cells and the red color of the solution is so intense that the "ghost" cells are completely obscured (Fig. 1). The percentage volume of opaque unhemolyzed erythrocytes in each pipette after centrifugation may also be recorded in comparison with the packed cell volume of the whole blood, but owing to certain unavoidable errors in dealing with a small amount of blood such as recommended for the microfragility test here described, the observation of the cell volume alone does not yield consistent results of any particular value.

Attempts at exact quantitation of hemolysis by use of a colorimeter employing standard solution of hemoglobin for comparison have been made by

some observers, particularly Lapieque and Vast (1899), Lepeschkin (1932), and Waugh and Asherman (1938), but such procedures appear to be entirely unnecessary inasmuch as the quantitative estimation of liberated hemoglobin is not the primary object of the test. For practical clinical purposes it is quite sufficient to indicate the range of hemolysis by simply noting the concentrations of salt solution in which hemolysis begins and ends, in the manner indicated in Table II.

Degrees of Hemolysis

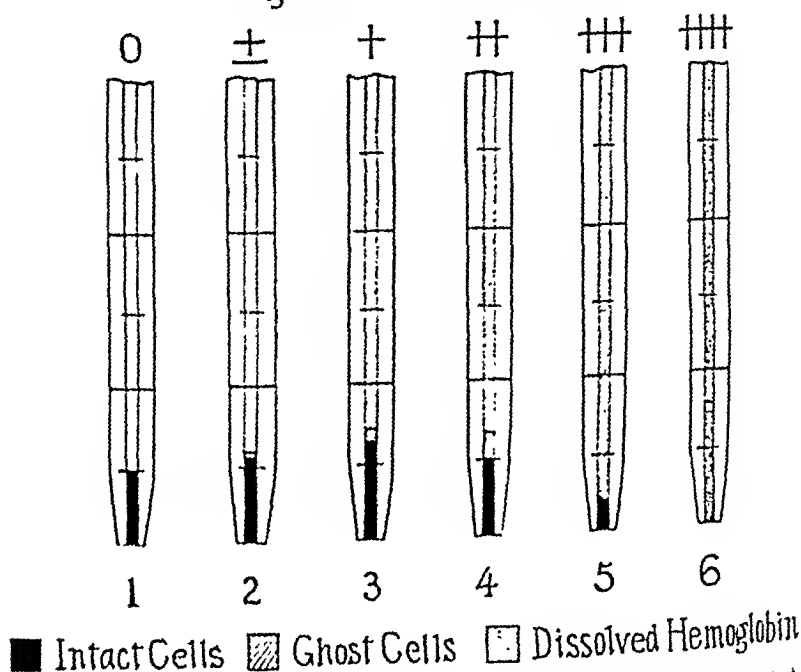


Fig. 1.—Six combination microhemipipettes (1-6) used in the microfragility test. No the absence of hemolysis in the first pipette and complete hemolysis in the last pipette. The maximum cell volume is obtained in pipette 3 and the initial hemolysis is seen in pipette 2.

COMMENT

The microfragility test here described is an attempt to simplify the more commonly used method for measuring the resistance of erythrocytes to hypotonic solutions of sodium chloride. Three distinct advantages of this test are: (1) simplicity in technical manipulation; (2) small amount of blood required for the test; and (3) accuracy attained by the use of graduated microhemipipettes which facilitate the exact observation not only of the degree of hemolysis in the supernatant fluid, but also of the volume of hemolyzed cells which overlies the column of opaque intact erythrocytes. The microfragility test may be most conveniently used in pediatric practice and in hematologic research on small experimental animals.

According to the results obtained from observations on a number of normal human beings, the microfragility test reveals the average initial hemolysis that occurs at the sodium chloride concentration of 0.435 per cent and the co

plete hemolysis at 0.330 per cent, giving an average of 0.1 per cent as a normal hemolysis span. Slight variations may be found from time to time, but under normal conditions the resistance of human erythrocytes against hypotonic salt solutions remains remarkably constant. Any significant deviation from the normal range mentioned, especially in the point of initial hemolysis, usually indicates the presence of erythrocyte anomaly and calls for repeated observations. A few examples of instances in which abnormal fragility has been found are summarized in Table III.

TABLE III
ABNORMAL FRAGILITY IN VARIOUS CONDITIONS

DISEASE	R.B.C. (MILLIONS)	HB. (GM. %)	PACKED CELL VOLUME (%)	CORPUSCLE VOLUME (CM)	ICTERIC INDEX (MURPHY UNITS)	INITIAL HEMOL- YSIS (% CONC. NaCl)	FINAL HEMOL- YSIS (% CONC. NaCl)
congenital hemo- lytic icterus (Remission)	3.75	10.2	30	88	30	0.450	0.375
catarrhal jaundice	4.92	12.9	37	85	120	0.435	0.255
leucemia, acute	1.56	4.4	18	125	2	0.405	0.315
anemia, post- hemorrhagic	3.54	6.7	22	69	2	0.403	0.330
rheumatic carditis	4.33	13.8	40	103	2	0.450	0.360
anemia, chronic infection	1.81	2.8	12	74	2	0.390	0.285
hemorrhagic dis- ease of newborn	6.21	18.9	62	100	15	0.480	0.345
hemorrhagic dis- ease of newborn	6.10	19.0	63	103	30	0.510	0.300
normal newborn	5.09	16.0	55	108	20	0.525	0.330

One of the most important clinical conditions in which the test proves to be most useful is in jaundice so frequently seen in various types of liver disease and blood dyscrasias. Since Chauffard in 1907 first demonstrated the increased fragility of erythrocytes in congenital hemolytic jaundice, numerous observers have confirmed this striking phenomenon. In one instance of this disease the microfragility test performed during a remission disclosed only a slight increase in erythrocyte fragility, namely, 0.450/0.375 per cent, indicating a definite shortening of the span of hemolysis. In a case of moderately severe catarrhal jaundice with pronounced bilirubinemia (van den Bergh 15 units) the initial hemolysis occurred at a normal concentration (0.435 per cent), but the hemolysis was not complete until the concentration reached an abnormally low level (0.255 per cent), indicative of a great prolongation of the hemolysis span. During the terminal stage of a case of acute leucemia with a marked anemia (erythrocyte count 1,560,000) and extreme leucopenia (leucocyte count 900), there was a slight increase in erythrocyte resistance (0.405/0.315 per cent). Practically identical results in fragility tests were obtained in a case of moderately severe posthemorrhagic anemia (erythrocyte count 3,540,000) in a 7-year-old child. In a case of rheumatic carditis in a quiescent stage erythrocyte fragility was slightly decreased (0.450/0.360 per cent), while in one instance of advanced rheumatoid arthritis with a pronounced anemia (erythrocyte count

1,810,000) an abnormal increase in erythrocyte resistance (0.390/0.285 per cent) was noted. In normal newly born infants it is customary to find a distinct increase in erythrocyte fragility (average 0.510/0.300), a phenomenon which satisfactorily explains the pathogenesis of physiologic jaundice of the newborn.

The most remarkable instance of greatly increased erythrocyte fragility was seen in a newborn infant with erythroblastosis fetalis (*icterus gravis neonatorum*) with a high icteric index (150 Murphy units). The microfragility test performed toward the end of the first twenty-four hours of life revealed

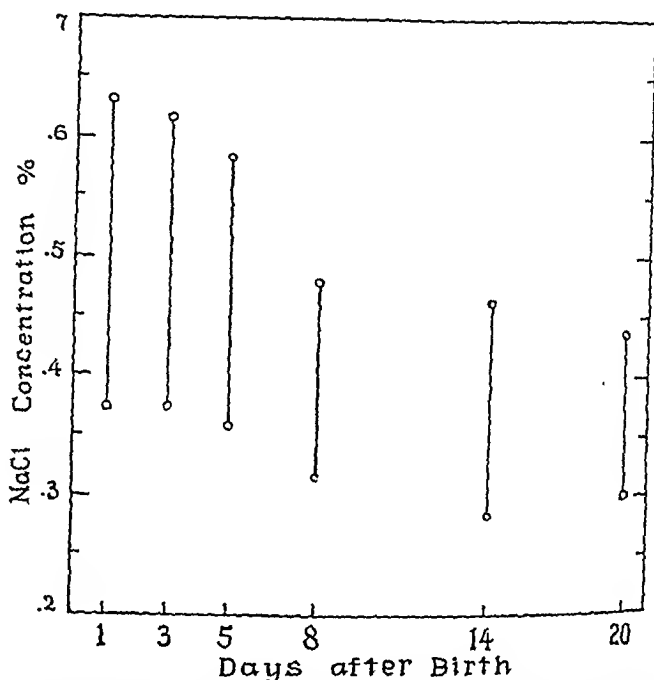


Fig. 2.—Hemolysis spans of erythrocytes in a case of erythroblastosis fetalis (*icterus gravis neonatorum*). Note increased fragility with prolonged hemolysis spans during the first five days of life and gradual return to the normal span upon recovery.

initial hemolysis at a concentration of 0.630 per cent and complete hemolysis at 0.375 per cent (Fig. 2). Upon repeating the test on subsequent dates, it was found that erythrocyte resistance gradually increased so that by the fourteenth day of life the cells began to hemolyze at a concentration slightly above the limits of normal range. Incidentally, it is to be noted that the gradual decrease in erythrocyte fragility in this infant coincided with the decrease in the percentage number of nucleated red blood cells, which on the first day of life was as high as approximately 75 per cent of the total nucleated blood cells.

These findings, though purely accidental, throw some light on the pathogenesis of extreme jaundice so characteristic of this disease. *Icterus neonatorum*, both physiologic and pathologic, has been the subject of repeated investigations by numerous observers, but as yet no conclusive evidence of its underlying etiology has been demonstrated. The accidental findings here recorded suggest very strongly the possibility of increased erythrocyte fragility, with consequent

bilirubinemia as being the fundamental cause of jaundice in the newborn. Moreover, in all probability, this increase in fragility is due to the rapid destruction of nucleated red blood cells which are so abundantly found in various organs as well as in the peripheral blood. The severity of jaundice appears to be in direct proportion to the increase in erythrocyte fragility, since erythrocyte fragility in normal newly born infants with physiologic type of icterus is only slightly increased and never to the extent seen in icterus gravis neonatorum. A more complete investigation of erythrocyte fragility in the newborn will be given in a later report.

FRAGILITY OF MAMMALIAN AND AVIAN ERYTHROCYTES

The fact that the resistance of erythrocytes against hemolytic agents is specific not only in each species of animals, but also in different individuals of the same species, was conclusively demonstrated by Hamburger (1902). The results of observations obtained by means of the microfragility test performed on the blood of mammals and birds, summarized in Table IV, reveal a great variation in erythrocyte fragility in the different species.

TABLE IV
MICROFRAGILITY TEST IN MAMMALS, PIGEON, AND MAN

SPECIES	AVERAGE NORMAL R.B.C. (MILLIONS PER C.MM.)	PACKED CELL VOLUME (%)	CORPUSCULE VOLUME (μ)	INITIAL HEMOLYSIS (% CONC. NaCl)	FINAL HEMOLYSIS (% CONC. NaCl)
Oat	16.00	55	34.5	0.615	0.480
Sheep	7.50	33	44.0	0.600	0.450
Rat	7.00	50	71.4	0.480	0.375
Rabbit	5.00	40	72.7	0.570	0.450
Mouse	10.00	80	80.0	0.535	0.330
Dog	6.50	55	84.6	0.450	0.360
Guinea pig	5.50	50	91.0	0.450	0.330
Pigeon	2.75	33	120.0	0.465	0.360
Man (infant)	6.00	55	90.0	0.510-0.450	0.315-0.300
Man (child)	4.00	35	88.8	0.435	0.330
Man (adult)	5.00	45	90.0	0.420	0.330

Data on the fragility tests in animals demonstrate the definite relationship existing between the volume as well as the shape of the red blood cells and their degree of resistance against hypotonic salt solutions. The fact that the fragility parallels the diameter of the cells was first noted by Vallery-Radot and Lehritier (1919), while Castle and Daland (1937) proved that differences in the susceptibility of various types of erythrocytes to hemolysis are due principally to differences in form and not to differences in osmotic behavior. Haden (1939), in numerous and painstaking studies of the peculiarities of erythrocytes in congenital hemolytic icterus, concludes that spherocytosis, which is so characteristic of this disease, is the essential factor in increased fragility.

In this connection it is also of interest to note the striking observation recorded by Rywosch (1907), namely, that the hemolytic action of hypotonic saline upon the resistance of mammalian erythrocytes exhibits a reverse of their resistance to saponin. Ponder, Saslow, and Yeager (1930), in re-examining a large number of resistance series of erythrocytes from various animals, have

proposed the hypothesis that in any hemolytic system certain physicochemical changes take place which affect the permeability of cell membranes, specific to the erythrocytes of each species and giving rise to different resistance series.

The increased fragility of erythrocytes in pigeons, in spite of their high mean corpuscular volume, requires separate consideration, inasmuch as their red blood cells are all nucleated.

SUMMARY

A new microfragility test, utilizing the same principle as that originally employed by Hamburger and used for clinical purposes by von Limbeck and by Ribierre, and later modified by numerous workers, particularly Giffin and Sanford, but greatly simplified and especially adapted for use in small infants and animals, is described.

As tested by this method, initial hemolysis of normal human erythrocytes is found to occur at an average of 0.420 to 0.435 per cent concentration of sodium chloride solution, while complete hemolysis is most frequently seen at 0.315 to 0.330 per cent concentration. The normal range or span of hemolysis is thus approximately 0.1 per cent concentration. The simplicity and accuracy of the test are the features especially adapted to routine hematologic examination.

A few illustrative examples of the results obtained by this method in various diseases characterized by abnormalities in erythrocyte fragility are reported. The striking increase in erythrocyte fragility in a case of erythroblastosis fetalis (icterus gravis neonatorum), as revealed by the microfragility test, suggests the fundamental mechanism in the causation of icterus in the newborn.

The normal hemolysis ranges of erythrocytes in a few mammals and pigeons, as measured by the microfragility test, are also recorded in this preliminary report.

REFERENCES

1. Beebe, R. T., and Hanley, E. P.: A Method for Measuring the Fragility of Erythrocytes in Salt Solution, *J. LAB. & CLIN. MED.* 21: 833, 1936.
2. Bigland, A. D.: The Fragility of the Red Blood Corpuscles in Physiological and Pathological States, *Quart. J. Med.* 7: 369, 1914.
3. Castle, W. B., and Daland, G. A.: A Function of Mammalian Erythrocytes to Hemolyze With Hypotonic Solutions: A Function of Differences Between Discoidal Volume and Volume of a Sphere of Equal Surface, *Arch. Int. Med.* 60: 949, 1937.
4. Chancel: Recherches sur la résistance des hématies, Thèse de Lyon, 1880.
5. Chauffard, A.: Pathogénie de l'ictère congénital de l'adult, *Semaine méd.* 27: 25, 1907.
6. Daland, G. A., and Worthley, K.: The Resistance of Red Blood Cells to Hemolysis in Hypotonic Salt Solutions of Sodium Chloride, Observations in Blood Disorders. *J. LAB. & CLIN. MED.* 20: 1122, 1935.
7. Duncan, J.: Beiträge zur Pathologie und Therapie der Chlorose, *Acad. de Soc. de Vienne*, p. 516, 1867 (Cited by Ribierre²³).
8. Fontaine, B. W.: A Simple Method of Determination of Erythrocyte Fragility of the Red Corpuscles, *J. A. M. A.* 77: 1022, 1921.
9. Giffin, H. Z., and Sanford, A. H.: Clinical Observations Concerning the Fragility of Erythrocytes, *J. LAB. & CLIN. MED.* 4: 465, 1919.
10. Guest, G. M., and Wing, M.: A Method for the Determination of Erythrocyte Fragility Using Van Allen Hematocrit Tubes for the Measurement of Changes in Volume of the Cells in Hypotonic Salt Solutions, *J. LAB. & CLIN. MED.* 24: 850, 1939.
11. Haden, R. L.: The Nature of Hemolytic Anemia, A Symposium on the Blood and Blood-Forming Organs, Madison, 1939, Univ. Wisconsin Press, p. 83.
12. Hamburger, H. J.: Osmotischer Druck und Ionenlehre in den medizinischen Wissenschaften, Wiesbaden, 1902, Bergmann, Bd. I, p. 183.

13. Harvey, W. F.: Studies in Method and Standardization of Blood Examination. IV. Estimation of Erythrocyte Fragility and a Normal Standard, *Edinburgh M. J.* 44: 100, 1937.
14. Hill, L. W.: The Resistance of the Red Blood Cells to Hypotonic Salt Solution in the Various Anemias, *Arch. Int. Med.* 16: 809, 1915.
15. Karsner, H. T., and Pearce, R. M.: The Relation of the Spleen to Blood Destruction and Regeneration and to Hemolytic Jaundice, *J. Exper. Med.* 16: 769, 1912.
16. Kato, Katsupi: Combination Microhemopipette, *J. LAB. & CLIN. MED.* 23: 980, 1938; Use of Combination Microhemopipette, With Special Reference to Determination of Sedimentation Rate, Packed Cell Volume and Icteric Index in Pediatric Practice, *Am. J. Dis. Child.* 59: 310, 1940; Micro Prothrombin Test With Capillary Whole Blood: A Modification of Quick's Quantitative Method, *Am. J. Clin. Path.* 10: 147, 1940.
17. Lang, G.: Ueber die Resistenz der rothen Blutkörperchen gegen hypotonische NaCl-Lösung bei Magenkrebs, *Ztschr. f. klin. Med.* 47: 153, 1902.
18. Lapique, L., and Vast, A.: Méthod colorimétrique pour apprécier la résistance globulaire, *Compt. rend. Soc. de biol.* 51: 366, 1899.
19. Leake, C. D., and Pratt, H.: The Resistance of Normal Human Erythrocytes to Hypotonic Saline Solutions, *J. A. M. A.* 85: 899, 1925.
20. Lepeschkin, W. W.: Fragility of Red Corpuscles and Its Determination in Clinical Work, *J. LAB. & CLIN. MED.* 17: 1250, 1932.
21. Malassez, L.: Les premières recherches sur la résistance des globules rouges, *Compt. rend. Soc. de biol.* 10: (s.2): 2, 1892.
22. McNeil, C.: The Resistance of Human Red Blood Corpuscles in Health and Disease to Hemolysis by Saponin, With a Comparison of Saponin and Hypotonic Salt Solution Hemolysis, *J. Path. & Bact.* 15: 56, 1911.
23. Ponder, E.: The Mammalian Red Cell and the Properties of Hemolytic Systems, Berlin, 1934, Borntraeger; The Measurement of Red Cell Volume. VI. The Different "Fragility" of the Red Cells of Various Mammals, *J. Physiol.* 83: 352, 1935.
24. Ponder, E., Saslow, G., and Yeager, J. F.: Studies on Kinetics of Hemolytic Systems, Farther Study of Series of Rywosch, *Biochem. J.* 24: 805, 1930.
25. Ribierre, P.: L'hémolyse et la mesure de la résistance globulaire; Application à l'étude de la résistance globulaire dans l'ictère, Thèse de Paris, 1903.
26. Rywosch, D.: Vergleichende Untersuchungen über die Resistenz der Erythrocyten einiger Säugetiere gegen hämolytische Agentien, *Pflüger's Arch. f. d. ges. Physiol.* 116: 220, 1907.
27. Silrette, H.: The Estimation of Red Cell Fragility: An Hematocrit Method, *J. LAB. & CLIN. MED.* 13: 662, 1928.
28. Simmel, H.: Die osmotische Resistenz der Erythrocyten, *Deutsches Arch. f. klin. Med.* 142: 252, 1923.
29. Smith, Theobald: The Pathological Effects of Periodic Losses of Blood—An Experimental Study, *J. M. Research* 12: 385, 1904.
30. Vallery-Radot, P., and Lheritier, A.: Parallélisme entre la résistance globulaire aux solutions chlorurées sodiques et la dimension de l'hématie chez les mammifères, *Compt. rend. Soc. de biol.* 82: 195, 1919.
31. Van Allen, C. M.: An Hematocrit Method, *J. LAB. & CLIN. MED.* 10: 1027, 1925.
32. Von Limbeck, R.: Grundriss der klinische Pathologie des Blutes, Jena, 1896, Gustav Fischer.
33. Vaquez, H.: Résistance des globules rouges, Congrès internat. de méd., Paris, Rapport Sect. d'anat. path., p. 334, 1900.
34. Waugh, T. R., and Chase, W. J.: A Combined Macroscopic and Microscopic Erythrocyte Fragility Technic (Modified Method of Simmel), *J. LAB. & CLIN. MED.* 13: 872, 1928.
35. Waugh, T. R., and Asherman, E. G.: The Use of an Index of Hemolysis in Expressing the Fragility of Erythrocytes, *J. LAB. & CLIN. MED.* 23: 746, 1938.
36. Wiseman, B. K., and Bierbaum, O. S.: A New Method for Determining the Fragility of Red Blood Cells, *Proc. Soc. Exper. Biol. & Med.* 29: 835, 1932.
37. Whitley, L. E. H., and Hynes, M.: Quantitative Estimation of Fragility of Red Corpuscles, *J. Path. & Bact.* 40: 219, 1935.

CHEMICAL

THE USE OF OSTEOTROPIC DYES IN A MODIFIED LINE TEST FOR VITAMIN D*

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THERE are three methods for the estimation of vitamin D: the estimation of the ash content of bones (humerus or femur);¹ the line test;² and the x-ray examination of bones.³ The determination of ash content has never received wide acceptance, as it is a much more tedious process than the line test. Hume and co-workers⁴ evaluated this technique and found that when the percentage of ash was plotted against the logarithms of the doses, a linear relationship between the two variables was revealed, showing that as the doses were increased in geometrical progression the calcification increments advanced in arithmetical series. It is an excellent method but, for practical reasons, it is difficult to apply.

The x-ray technique was studied by Bourdillon and associates,⁵ who attributed such an increased accuracy to the method that they listed twelve different degrees of healing in rachitic rats. Compared to the bone ash method, radiographic comparison is equally accurate, and because it is easier to apply it is the better of the two methods.

The line test is the more generally accepted procedure because it is inexpensive, requires no special apparatus, and presents no technical difficulties. Bills and others⁶ in a "critique" of the line test, state that by using 100 animals the error is reduced to 4 per cent. The line test depends upon the conversion of silver phosphate to black colloidal silver following exposure to light. Bills lists the different types of healing as the epiphyseal, in which new calcium is laid down in a narrow line at the distal edge of the cartilage; the metaphyseal, in which deposition occurs at the metaphysis; and the diaphyseal, in which the first calcification is diaphyseal and contiguous with the trabeculae that remain after rickets develops. The latter is useless for the line test. The line test is most delicate, since it detects very early stages of healing which x-ray will not show. Bone, whether newly deposited or not, gives the reactions involved in the silver method. Deposition of bone when it does occur is not necessarily along the line of calcification examined in the line test. These facts frequently complicate the accurate diagnosis of a bone section.

As uncalcified tissue will not stain with hematoxylin, and calcified tissue will, a method of hematoxylin staining has been used, but, again, there is no differentiation between the newly formed and the old bone.

Gottlieb⁷ suggested the use of alizarin and purpurin in the staining of newly formed bone. The suggestion was made that the mechanism involved the local

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concentration of calcium ions. The use of alizarin and of purpurin was studied by Cameron,⁸ who injected sodium alizarin sulfonate in a 2 per cent solution. Bones started developing the color in five minutes and became most intensely colored in twelve hours. Anthrapurpurin and purpurin gave less pronounced results. Cameron used the method in studying fracture callus in the rat. The callus was unstained at ten days, deeply stained from ten to sixty days, and practically unstained thereafter. Alizarin was also used to stain the calcified areas produced by hypervitaminosis D. A method used by Brash⁹ consists of feeding madder and then stopping the administration so that new bone growth is white, while all bone formed during the madder feeding period is stained from pink to red. This seemed to be the clue to a highly specific line test modification. The mechanism of the osteotropic dye is suggested by Ercoli¹⁰ to be an adsorption phenomenon. The sodium alizarin sulfonate is irreversibly fixed and a red complex is formed. Phosphate ion is liberated in this mechanism.

EXPERIMENTAL

Sodium alizarin sulfonate, a water-soluble derivative of alizarin, is recommended by Cameron⁸ for its osteotropism. We administered 1 c.c. of a 2 per cent solution to 60 Gm. rats and found we were above or at the fatal dose. The dosage was cut to 0.2 c.c. of 2 per cent sodium alizarin sulfonate and found to be harmless, but it gave no distinct coloration to the bones. The compound showed no marked osteotropism, probably because of its water solubility.

Next, a 2 per cent suspension of alizarin in water was used. One cubic centimeter each was injected intraperitoneally into 20 rats. No bone coloration was observed in any of the supplemented animals. Here the complete insolubility of the alizarin prevented its being effective. Examination of the peritoneal cavities of these animals showed unabsorbed dye in large amount.

Finally, sodium alizarinate was used in a 1 per cent solution in water at a pH of 8.0. In one experiment of twenty-four-hour duration, 1 c.c. of the 1 per cent sodium alizarinate solution was injected intraperitoneally. At the same time, supplements containing varying amounts of vitamin D in oil were given orally. Twenty-four hours later the animals were killed, and the radii and ulnae were removed, split lengthwise, and examined microscopically. The negative controls showed no coloration or a faint pink tinge, indicating the absence of new bone formation. If any coloration is seen, it is generally disseminated, coloring the entire bone and assuming no definite pattern, a state such as occurs following new calcium deposition. Three rats receiving a total of 0.3 units of vitamin D showed 1+ and 2+ healing by our scale. The calcium deposition may occur along the line of calcification in the epiphysis or the diaphysis, and yet distinct coloration, which can be used in assigning a value of healing, is observed. Three rats receiving a total of 1 unit of vitamin D showed a 2+ and 3+ healing. As the bones are semitransparent, it is possible to observe macroscopic differences in color intensity without cutting them. It is possible that a twenty-four-hour test can be developed, using macroscopic examination.

Ten rats were used in a two-day test. One cubic centimeter of 1 per cent sodium alizarinate was given intraperitoneally on the first day to all rats. Six-

tenths unit of vitamin D was given to the supplemented animals in two doses of 0.3 unit each. Forty-eight hours after the first dose of vitamin D was given, the animals were killed and the bones were examined. Values of 3+ and 4+ were assigned to the degree of healing seen.

Arbitrary values are assigned to divide into six divisions, from 0 to 6, the range of healing from absence of coloration with no vitamin D to the coloration of the bone at complete healing. A value of 6 indicates complete healing. Chart I illustrates these stages of healing.

TABLE I
COMPARISON OF SILVER AND ALIZARIN LINE TESTS
Two-Day Test
(Numbers indicate the degree of healing.)

NEGATIVE CONTROLS		STANDARD 0.95 UNIT DAILY		STANDARD 1.9 UNITS DAILY	
SILVER	ALIZARIN	SILVER	ALIZARIN	SILVER	ALIZARIN
0	0	2	3	3	5
0	0	2	3	3	5
0	0	1	2	2	5
0	0	2	3	0	4
0	0	3	3	2	5
1	0	3	3	3	4
0	0	1	2		
UNKNOWN 0.1 C.C. CALCULATED 0.31 UNIT DAILY		UNKNOWN 0.3 C.C. CALCULATED 0.93 UNIT DAILY		UNKNOWN 0.6 C.C. CALCULATED 1.9 UNITS DAILY	
SILVER	ALIZARIN	SILVER	ALIZARIN	SILVER	ALIZARIN
0	1	2-3	3	4-5	5
0-1	1	0-1	4	4-5	5
1	1	2	3	3-4	5
0	1	4	4	4	5
0-1	1	0	3	2	4
0	1	2	3	5	5
1	1	2	3	2	4

A series of tests was conducted, using both the silver staining² and the alizarin techniques. The results are recorded in Table I. This table shows a more uniform result where alizarin was used. In this group the sodium alizarinate was given intraperitoneally on the date of the first dosage with vitamin D, and the bones were examined forty-eight hours later.

The second series of sodium alizarinate animals was given intraperitoneal dye (1 c.c. of 1 per cent) after the third dosage of vitamin D in a series of five daily doses. The bones were removed and examined on the sixth day. An improved coloration was noted which, compared to the silver line test, gave the results in Table II.

A third series for comparison was conducted by giving the sodium alizarinate (0.3 c.c. of 1 per cent) on three different occasions: at the time of the first vitamin D supplement, on the third day, and when the final dose (fifth day) of vitamin D was given. The group showed no material improvement over the second series in accuracy, and the additional administrations are, therefore, regarded as unnecessary. The results of this smaller group are recorded in Table III.

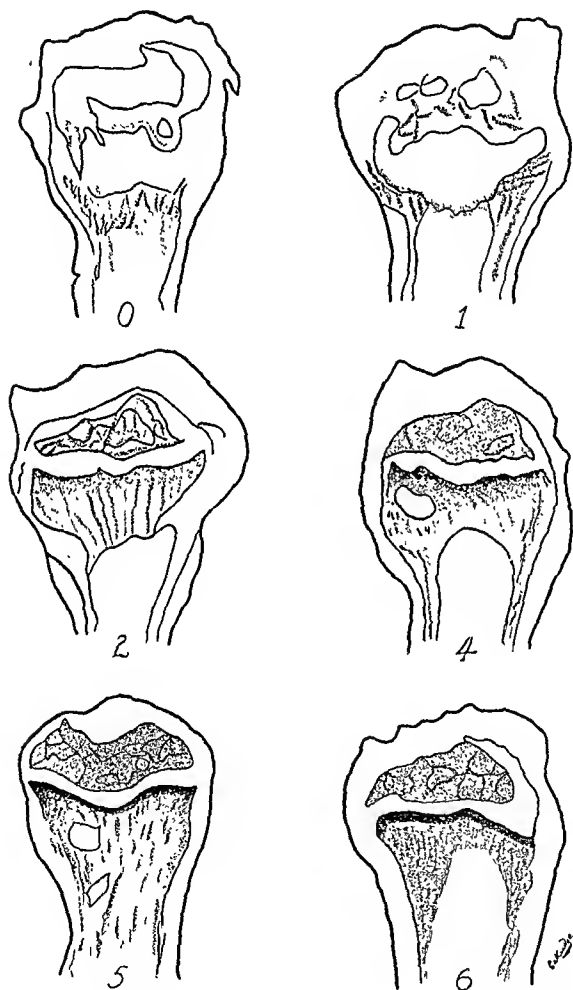


Chart 1.—Alizarin line test. Numbers indicate the degree of healing. Number 6 indicates complete healing.

TABLE II
COMPARISON OF SILVER AND ALIZARIN LINE TESTS
Five-Day Test
(Numbers indicate the degree of healing.)

NEGATIVE CONTROLS		STANDARD 0.95 UNIT DAILY		STANDARD 1.9 UNITS DAILY	
SILVER	ALIZARIN	SILVER	ALIZARIN	SILVER	ALIZARIN
0	0	3	5	4-5	6
0	0	2	5	5	6
0	0	1	4-5	3	6
1	0	3	4	5	6
0	0	0	5	4	6
1	0	3	4	3	5
UNKNOWN 0.1 C.C. CALCULATED 0.31 UNIT DAILY		UNKNOWN 0.3 C.C. CALCULATED 0.93 UNIT DAILY		UNKNOWN 0.6 C.C. CALCULATED 1.9 UNITS DAILY	
SILVER	ALIZARIN	SILVER	ALIZARIN	SILVER	ALIZARIN
0	1-2	3-4	4-5	4-5	6
1-2	2	2	5	5	6
0-1	2	3	5	3-4	6
1-2	3	2	4-5	5	6
2	2	4	4	5	6
1-2	2	1	4	3-4	5

TABLE III
COMPARISON OF SILVER AND ALIZARIN LINE TESTS
Five-Day Test, Alizarin Injected for Three Days
(Numbers indicate degree of healing.)

NEGATIVE CONTROLS		STANDARD 0.95 UNIT DAILY	
SILVER	ALIZARIN	SILVER	ALIZARIN
0	0	3	5
1	0	3-4	4-5
0	1	2-3	5
1-2	0	3	4-5
1-2	0	4	5
0	0	3	5
1-2	0	2	5
1	1	3	4-5

DISCUSSION

Cameron⁸ has pointed out that alizarin is a nearly specific stain for calcium. It reacts readily in vitro or in vivo with recently deposited calcium salts, either normal or pathologically formed, but it fails to stain older deposits. Silver nitrate is not a test for calcium or phosphate; the black reaction is given by a variety of solids, the anion being more important than the cation. As pointed out by Bills and associates,⁶ deposition of calcium following administration of vitamin D may not occur along epiphyseal or diaphyseal lines, and, if it does not, the silver line test is useless. Hematoxylin does not stain calcium salts, though it often identifies areas in which changes favorable to the deposition of calcium salts are taking place. Alizarin stains only newly formed bone. It stains these calcium deposits whether they are epiphyseal, diaphyseal, or metapiphyseal. It follows that greater specificity, greater applicability, and greater speed result from the use of alizarin.

SUMMARY

A modified line test involving the use of an osteotropic dye, alizarin, is introduced. Greater specificity, greater applicability, and greater speed result from its use.

REFERENCES

1. Bethke, R. M., Steenbock, Harry, and Nelson, M. T.: Fat Soluble Vitamins, *J. Biol. Chem.* 58: 71, 1923.
2. McCollum, E. V., Simmonds, Nina, Shipley, P. G., and Park, E. A.: A Delicate Biological Test for Calcium Depositing Substances, *J. Biol. Chem.* 51: 41, 1922.
3. Poulsson, E., and Löffenskiöld, H.: Quantitative Determination of Vitamin D, *Biochem. J.* 22: 135, 1928.
4. Hume, E. M., Pickersgill, R., and Gaffiken, E. L.: The Relation Between Graded Doses of Vitamin D and Ash Content of Bones, *Biochem. J.* 26: 488, 1932.
5. Bourdillon, R. B., Bruce, H. M., Fischman, C., and Webster, T. A.: Quantitative Estimation of Vitamin D Radiography, *Med. Res. Council, Special Report No. 158*, London, 1931.
6. Bills, C. E., Honeywell, E. M., Wiriek, E. M., and Nusameier, G. A.: A Critique of the Line Test for Vitamin D, *J. Biol. Chem.* 90: 619, 1931.
7. Gottlieb, B.: Die Vitale Färbung der Kalkhaltigen Gewebe, *Anat. Anz.* 46: 179, 1914.
8. Cameron, G. R.: The Staining of Calcium, *J. Path. Bact.* 33: 929, 1930.
9. Brash, J. C.: Developmental Mechanism of Bone, *Edinburgh M. J.* 41: 305, 1934.
10. Ercoli, N.: Osteotropism of Pharmaceutical Substances, *Bull. soc. ital. biol. sperm.* 13: 755, 1938.

RECOVERY OF CARBON TETRACHLORIDE FROM TISSUE*

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THE difficulty encountered by Gettler and Siegel¹ in the recovery of pure carbon tetrachloride after addition to tissue seemed to me to be due to the heating of the carbon tetrachloride in the presence of tissue. The first step in their method is steam distillation. While they were able to recover the carbon tetrachloride by rectification after steam distillation of carbon tetrachloride in water, the method failed when applied to carbon tetrachloride added to tissue. In the latter case a part of the carbon tetrachloride was converted to chloroform. To obviate heating, the method here described was devised.

THE APPARATUS

The apparatus is shown in Fig. 1. The ground glass joints connecting *A* and *B*, and *B* and *C* are 45-50. The tube *D* is a Gettler collection tube^{1, 2} sealed into the bottom of *C*. This collection tube is 13 cm. long, has a diameter of 0.7 cm., and a capacity of 3 c.c. graduated in twentieths. By means of rubber vacuum tubing *E* is connected to two glass traps in series. The second trap is connected through an ordinary closed-end mercury manometer to a vacuum pump.

Flask *A* is supported by a condenser clamp at the neck, and *C* is prevented from falling by copper wire fastened tightly around the neck of *C* and coiled loosely about the horizontal portion of *B*. The usual glass hooks sealed in above and below the glass joint and rubber bands may also be used.

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PROCEDURE

The distillation flask *A* was immersed to the bottom of the neck in dry ice and acetone contained in a large Dewar flask. Some glass wool had been placed in the Dewar to prevent spontaneous overflowing. Half of the tissue, ground lamb's brain, was introduced into *A*. The temperature of the carbon tetrachloride was taken (room temperature). It was introduced by means of a pipette graduated in hundredths. The second half of the tissue was then introduced. After the ground glass joints had been greased with Lubriscal, *B* and *C* were attached, and the stopcock in the side arm of *B* was closed, *E* was connected to the first trap, and the tissue and carbon tetrachloride were allowed to freeze for the length of time indicated in Table I.

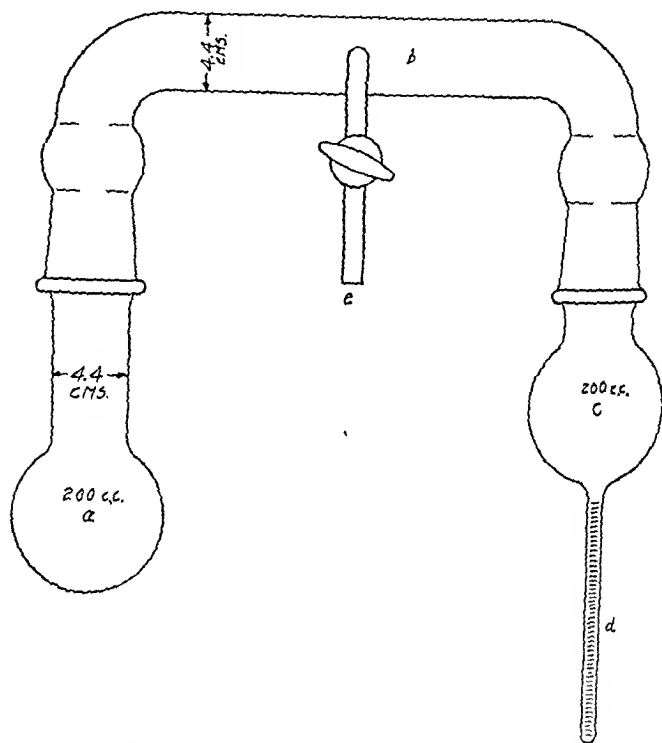


Fig. 1.—Apparatus for distillation and measurement of recovered liquid.

Each of the two traps was immersed in a Dewar containing dry ice, acetone, and glass wool; the stopcock in the side arm of *B* was opened and the vacuum pump was turned on. The pump was allowed to run for about ten minutes after the minimum pressure was attained, which averaged about 0.5 mm. The stopcock was closed and the rubber tubing was disconnected.

The large Dewar containing dry ice and acetone was shifted from *A* to *C*, the level of the liquid being adjusted to the midpoint of the bulb *C*. The flask *A* was immersed to the bottom of the neck in water at about 25° C., and the distillation was allowed to run for the length of time indicated in Table I. At the end of the distillation the stopcock was opened to allow the pressure inside to come to that of the atmosphere, and then closed. The apparatus was then raised so that only the lower half of the collection tube *D* was immersed in the dry ice

and acetone. When all the solid in *C* had melted, *C* was disconnected at the ground glass joint, stoppered with a cork, and the collection tube immersed in water and kept at the temperature at which the carbon tetrachloride had been measured until no change in volume could be observed. The amount of the distilled water above the carbon tetrachloride varied from 0.05 to 2 c.c. In spite of scrupulous cleaning, first with soap and water and then with chromic acid or concentrated nitric acid and drying in an oven, the bulb *C* always contained droplets of moisture at the end of the melting. After water was dropped into *C* to wash this moisture into *D*, additional carbon tetrachloride was sometimes recovered.

TABLE I

NO.	TISSUE (GM.)	CCl ₄ (C.C.)	FREEZ- ING TIME (MIN.)	DISTIL- LATION TIME (MIN.)	RECOV- ERY (C.C.)	RECOV- ERY %	BOILING POINT ORIGI- NAL	BOILING POINT RECOV- ERED MA- TERIAL	REFRACT- IVE INDEX ORIGINAL	REFRACT- IVE INDEX RECOV- ERED MATERIAL
1	75	1.00	120	90	0.95	95.0	76.6	76.6	1.4607	1.4603
2	50	1.00	60	135	0.95	95.0	76.6	76.6		1.4602
3	50	1.00	45	60	0.93	93.0	76.7	76.4		1.4607
4	50	1.00	120	100	0.90	90.0	76.6	76.6		1.4602
5	50	1.00	60	155	0.93	93.0	76.6	76.6		1.4603
6	50	1.00	130	45	0.95	95.0	76.0	76.6		1.4603
7	45	1.00	50	60	0.93	93.0	76.4	76.4		1.4607
8	50	1.00	80	80	0.95	95.0	76.6	76.6		1.4605
9	50	0.50	65	82	0.40	80.0	76.6	76.5		1.4603
10	50	0.50	60	60	0.40	80.0		76.3		1.4607
11	50	0.75	110	90	0.70	93.3	76.6	76.7		1.4610
12	50	0.70	60	60	0.63	90.0	76.6	76.7		1.4608
13	50	0.70	75	60	0.65	92.9	76.6	76.6		1.4600
14	100	1.00	135	155	0.95	95.0	76.6	76.6		1.4610
15	100	1.00	60	120	0.95	95.0	76.6	76.6		1.4609
16	100	0.70	60	115	0.64	91.4	76.6	76.5		1.4609
17	100	0.70	05	140	0.61	87.1	76.7	76.2		1.4606
18	100	0.50	60	105	0.45	90.0	76.7	76.7		1.4605
19	100	0.50	80	90	0.45	90.0	76.6	76.8		1.4607

The recovery in cubic centimeters and percentage is given in Table I. The purity was determined by the microboiling point, Siwoloff's method,³ and the refractive index of the carbon tetrachloride layer. The boiling point of the original carbon tetrachloride was determined at as near the same pressure as was practicable. The refractive indices were corrected for temperature by applying the general rule: refractive index decreases 0.0004 per degree Centigrade rise above 20°.

All recovery samples and the original were tested for chloroform in carbon tetrachloride by the cyclohexanol, alpha-naphthol, potassium hydroxide method.⁴ Each gave a negative result.

A small amount of frost always appeared in the glass traps. The rate of evaporation on warming to room temperature suggested water. On only one occasion was there a sufficiently large drop for a boiling point determination. In this case it was water.

Putrefaction of the tissue apparently does not affect the purity of the distillate. In determinations 1, 4, and 17 in Table I, the tissue was very putrid, and in many other cases it was several weeks old.

DISCUSSION

I believe some carbon tetrachloride is lost during its introduction into the distilling flask. There is always a strong odor of carbon tetrachloride during this process. Also, while calibration of the collection tube against the pipette using mercury shows a check to 0.01 c.c., the transfer of 1 c.c. of carbon tetrachloride from the pipette to the collection tube at room temperature shows a loss of 0.025 c.c. It may be possible that the carbon tetrachloride present in tissue poisoned in vivo may be frozen at a low temperature, the tissue ground and introduced before thawing sets in, and the carbon tetrachloride recovered with less loss. It is also possible that some carbon tetrachloride is vaporized during the melting, or that some remains in the distillation flask.

The method should be adaptable for use in isolating ether or any other liquid immiscible with water which boils below 100° C. from tissue or other similar mixtures.

SUMMARY

A low temperature, low pressure distillation method for the recovery of carbon tetrachloride after addition to tissue is described.

REFERENCES

1. Gettler, Alexander O., and Siegel, Henry: Isolation From Human Tissues of Easily Volatile Organic Liquids and Their Identification, *Arch. Path.* 19: 208, 1935.
2. Gettler, Alexander O., and Siegel, Henry: Quantitative Isolation of Ethyl Alcohol From Tissues of Alcoholics, *Am. J. Clin. Path.* 7: 85, 1937.
3. Siwoloboff, A.: Ueber die Siedepunktbestimmung kleiner Mengen Flüssigkeiten, *Ber. d. deutsch. chem. Gesellsch.* 19: 795, 1886. The modification used was that described in Shriner, Ralph L., and Fuson, Reynold C.: *The Systematic Identification of Organic Compounds*, ed. 2, New York, 1940, John Wiley & Sons, Procedure C, p. 93.
4. Weber, Hans H.: Zur Methodik der Analyse technischer Lösungsmittel, *Chemiker-Zeitung* 61: 807, 1937.

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A SIMPLIFIED MICRO TEST OF PLASMA PROTHROMBIN*

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A SIMPLIFIED micro test of plasma prothrombin based on the principles of the Smith test has been found to be as accurate as the Smith test used by us for the determination of the prothrombin clotting activity of the blood. A useful micro Quick test has been devised by Kato¹ but it is as complicated as the Quick method² because of the number of steps and ingredients involved in the test. However, as in the Smith method,³ the simplified micro test as herein suggested depends upon the use of only blood and thromboplastin. Utilizing the principle of the Smith test, the micro test was investigated and was found to be surprisingly accurate and simple to perform. The test was done on patients suspected of having a hypoprothrombinemia and checked by the Smith method. The results were recorded in per cent of prothrombin clotting activity (Tables I and II).

The micro test is performed in the following manner: A puncture wound is made in the ear, finger, or heel, and with a Kato microhemopipette 30 to 40 c.mm. of blood are drawn into the tube. This is transferred immediately to a watch glass and mixed with 10 c.mm. of thromboplastin.† The glass is tilted back and forth until a clot is formed. The time recorded for the formation of the clot is divided into the control prothrombin clotting time obtained in the same manner from a normal individual. The result is the per cent of prothrombin clotting activity of the patient. Dangerous levels are those below 70 per cent. Hemorrhage may occur when these levels are reached. The time found by this method is usually five to eight seconds longer than the time by the Smith test. However, the percentage result is as accurate as in the Smith method. The only difficulty encountered in the micro test is the determination of the end point of the clot. With practice the exact end point can be readily determined.

The advantages of the micro method are obvious. It does not require a venipuncture and necessitates very little equipment and time to perform. Since venipuncture is unnecessary, it may be done on infants without difficulty. It

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†Thromboplastin is obtained from rabbit brains or lungs. Approximately 25 Gm. of the material is macerated in a mortar and mixed with about 100 c.c. of physiologic saline solution. This is allowed to stand in the icebox for three hours. The supernatant fluid is decanted and contains the active thromboplastin. The solution is kept in the icebox and is warmed to room temperature before using. If time for a normal individual is less than twenty seconds, the thromb illuded with saline so that the normal prothrombin time will be econds. The activity of the thrombo- plastin decreases daily; thus for the sample of thromboplastin used must be made on a normal st is performed on the patient.

TABLE I

COMPARATIVE PLASMA PROTHROMBIN VALUES FOR THE SMITH AND THE MICRO METHOD TAKEN UPON NORMAL INDIVIDUALS USING THE SAME THROMBOPLASTIN SOLUTION

Approximately 5 to 8 seconds difference occurs between the times of the two methods, but the percentage result is accurate for each method.

PATIENT	PROTHROMBIN CLOTTING ACTIVITY	
	SMITH METHOD	MICRO METHOD
1 Normal	26 sec. or 100%	33 sec. or 100%
2 Normal	26 sec. or 100%	34 sec. or 100%
3 Normal	28 sec. or 100%	34 sec. or 100%
4 Normal	30 sec. or 100%	35 sec. or 100%
5 Normal	31 sec. or 100%	37 sec. or 100%
6 Normal	30 sec. or 100%	37 sec. or 100%
7 Normal	30 sec. or 100%	36 sec. or 100%

TABLE II

COMPARATIVE PLASMA PROTHROMBIN VALUES IN 8 PATIENTS SUSPECTED OF HAVING A HYPOPROTHROMBINEMIA

The per cent plasma prothrombin activity as determined by the micro method is found to coincide with the clotting activity as determined simultaneously by the Smith method.

PATIENT AND DIAGNOSIS	PROTHROMBIN CLOTTING ACTIVITY	
	SMITH METHOD	MICRO METHOD
1 Choledocholithiasis	43 sec. or 72%	50 sec. or 70%
2 Choledocholithiasis	38 sec. or 71%	46 sec. or 74%
3 Choledocholithiasis	36 sec. or 84%	40 sec. or 80%
4 Choledocholithiasis	30 sec. or 100%	36 sec. or 100%
5 Cholecystitis	30 sec. or 88%	38 sec. or 88%
6 Cholecystitis	25 sec. or 84%	30 sec. or 83%
7 Carcinoma of pancreas	32 sec. or 79%	38 sec. or 80%
8 Cholelithiasis	24 sec. or 86%	32 sec. or 87%

may be done as quickly as a leucocyte or erythrocyte count, and in accuracy compares favorably with the Smith test.

The microhemopipette of Kato may be purchased from the Fisher Scientific Co., Pittsburgh, Pa.

REFERENCES

1. Kato, Katsuji: Microprothrombin Test With the Use of Capillary Whole Blood. A Simplified Modification of Quick's Quantitative Method, *Am. J. Clin. Path.* J. A. M. A. 114: 750, 1940.
2. Quick, A. J.: The Nature of Bleeding in Jaundice, *J. A. M. A.* 110: 1658, 1939.
3. Smith, H. P., Ziffren, S. E., Owen, C. A., and Hoffman, G. R.: Clinical and Experimental Studies on Vitamin K, *J. A. M. A.* 113: 380, 1939.

CONSISTENT BLOOD SUGAR VALUES OBTAINED FROM RABBIT HEART BLOOD SAMPLES*

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THE use of the rabbit for blood sugar estimations has often been questioned. Still the animal has been used, and in most cases has furnished the required information even if the data obtained were handled conservatively. Certain objections have remained, nevertheless, and in particular the influence of excitement. Even the trained animal becomes excited during ear vein puncture for peripheral blood; and in taking heart blood under ordinary precautions, excitement nearly always occurs and internal injury frequently takes place. In order to overcome these and other well-known objections, the following technique was put into operation, and the normal blood sugar values obtained (Hagedorn-Jensen¹) were subjected to statistical analysis.

TECHNIQUE

No anesthetic or assistance is needed in carrying out the technique, but a quiet room and a rabbit board of adequate length are essential for success.

The rabbit is gently lifted by its ears onto the lower short edge of the board and is held in an upright position (Fig. 1) until loops are placed on its extremities. After its hind legs are tied close, the rabbit is slowly lowered until its back rests on the board (Fig. 2). The forelegs are slowly drawn in the direction of the head and tied. Tying is necessary to keep the animal from rolling over. These steps are facilitated if the grip on the rabbit's ears is maintained with one hand until the other has completed the tying. The animal may be kept quiet for hours if its nose is stroked at intervals, or when deemed necessary.

Heart blood should be taken only after the rabbit has been resting on its back a few minutes. A syringe with a No. 19 or 20 gauge needle has been found best. The needle is introduced from the left costal region, and the blood is withdrawn slowly. When samples are taken at widely separated intervals, the rabbit may be lifted from the board and placed in a box until needed again, without removing the ties from the extremities.

EXPERIMENTS AND RESULTS

Blood sugar estimations were made on samples procured at the following intervals: 15, 30, 60, 120, 180, and 240 minutes. Two series of experiments were done. In one series, including 25 animals, the rabbit was kept on the board in the quiet state for the entire period of four hours. In the other series, 75 animals, the rabbit was placed in a box between the intervals. The latter experiments permitted study of the effect change in position would have on blood sugar values.

*From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago.
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Table I includes the mean values (m) and their respective standard deviation (σm) for the experiments during which the animal was kept supine for four hours, as well as when it was placed in a box between the time intervals. For the six intervals the mean values range between 74.12 and 77.00 mg. per cent in the first series, and between 77.70 and 81.15 mg. per cent in the second.



Fig. 1.

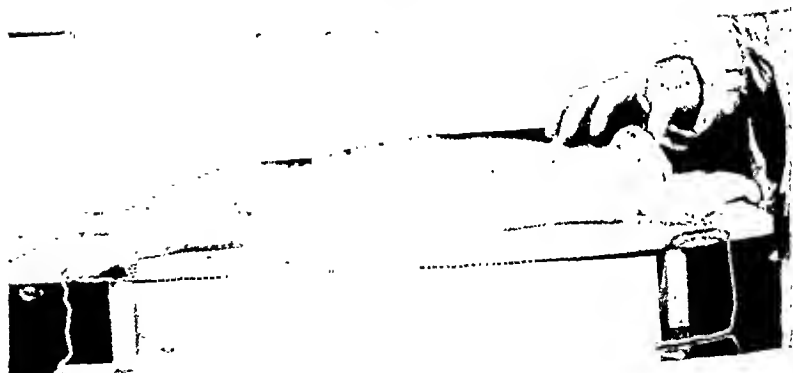


Fig. 2.

The greatest obtained difference, therefore, is 2.88 and 3.45, respectively. The mean of the six means of the former group is 75.45, and of the latter, 79.36, and their difference is 3.89.

DISCUSSION

Statistical study of the blood sugar values revealed fair agreement. The means obtained in the series of experiments when the animal was kept supine for four hours gave obtained differences which fell well within the range of error of the blood sugar method. This was also true when one treated similarly

the means obtained in the series when the animal was placed in a box between the intervals. Comparison of the two series indicated that one gains very little, if anything, by keeping the animal supine for a period of four hours.

TABLE I

BLOOD SUGAR VALUES IN MILLIGRAMS PER CENT OBTAINED BY THE HAGEDORN-JENSEN METHOD

INTERVALS IN MINUTES	25 RABBITS SUPINE FOR 4 HOURS		75 RABBITS NOT SUPINE BETWEEN INTERVALS	
	M	σM	M	σM
15	74.12	± 4.80	77.70	± 6.20
30	75.00	5.17	78.30	5.60
60	77.00	7.18	79.80	7.63
120	74.67	3.75	81.15	5.60
180	75.50	4.20	79.70	5.83
240	76.40	5.87	79.50	6.24

Several advantages are observed in the technique described. Skill entirely eliminates excitement in the animal. The method of handling, omission of an anesthetic, the facility with which heart blood may be taken in adequate quantities, all help furnish more accurate blood sugar values. With care the animal may be used again and again.

CONCLUSIONS

A technique is described which permits heart blood to be taken from rabbits without the need of an anesthetic or assistance and which renders consistent blood sugar values.

REFERENCE

1. Hagedorn, H. A., and Jensen, J. C.: The Quantitative Determination of Blood Sugar, *Biochem. Ztschr.* 135: 46, 1923; 137: 92, 1923.

EFFECT OF INGESTED IRON ON TESTS FOR OCCULT BLOOD IN STOOLS*

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IT IS not infrequently important to determine whether a patient, who is receiving iron by mouth to correct an anemia due to gastrointestinal hemorrhage, is still losing detectable amounts of blood. Tarry stools are not significant while he is ingesting iron. Inquiries, therefore, were instituted among a number of pathologists and laboratory workers to determine whether the usual chemical tests for occult blood in the stools were reliable under these conditions. The diversity of opinions suggested that actual experiment was indicated.

Since it has been found that large amounts of red meat in the diet may give a falsely positive benzidine test for occult blood in the stools, even though the guaiac test usually remains negative, seven persons were first put on a meat-free diet. After several days their stools were tested and found to give no

*From the Springfield Hospital, Springfield.
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reaction for occult blood with either benzidine or guaiac reagents.* Each individual was maintained on the same diet and given in addition 12 grains daily of ferrous sulfate by mouth. As soon as the stools became tarry the benzidine and guaiac tests were again applied. To our surprise the guaiac tests were repeatedly positive and the benzidine tests were consistently negative so long as the ferrous sulfate resulted in tarry stools. The iron was then discontinued and the stools were again tested for occult blood as soon as the tarry characteristics disappeared. Both benzidine and guaiac reagents once more gave negative results as before the iron was administered.

Nine other persons who were found to have both benzidine and guaiac-negative stools on the ordinary hospital diet were given daily 30 grains of ferrous sulfate by mouth. As soon as their stools became black they were found to give a positive reaction with the guaiac test but not with the benzidine test. After discontinuing the ferrous sulfate the stools of these patients gradually became guaiac negative, with the exception of one, as they lost their tarry appearance. This patient continued to produce stools that gave a positive reaction as late as five days after the tarry color had disappeared. The nine patients who had received double the usual therapeutic dose of ferrous sulfate did not produce guaiac-negative stools until several days after the excreta had lost their tarry color, whereas those who received only 12 grains daily gave guaiac-negative stools as soon as the stools regained their normal color.

As a check on these observations the following in vitro experiments were carried out. A solution of ferrous sulfate was made up from the tablets in therapeutic use, and the usual tests were carried out with both benzidine and guaiac reagents. Positive tests were again given by guaiac but not by benzidine. Our pathologist, Dr. F. D. Jones, suggested that the false positives with the guaiac reagent were due to tannin which gave rise to blue iron tannate. Accordingly, the benzidine reagent was used on a solution of ferrous sulfate and ferric chloride to which a small amount of tannic acid powder had been added. A characteristically blue reaction was then obtained as in the case of the guaiac reagent.

Because chlorophyll, which imparts the green color to many vegetables, is composed of pyrrole rings as is the hemoglobin molecule, it was thought that this substance might give rise to false tests for occult blood in the stools. Chlorophyll was, therefore, extracted with alcohol from various green plants and tested with both the guaiac and benzidine reagents. Both tests were negative.

CONCLUSIONS

Benzidine may give a falsely positive test for occult blood in the stools if the patient is eating considerable meat. This appears to be less likely with the guaiac test, which gives a falsely positive reaction for blood if the patient is receiving enough iron by mouth to produce tarry stools. The benzidine test is not affected by iron either in vivo or in vitro. Chlorophyll failed to give a positive reaction with either guaiac or benzidine in vitro.

*Benzidine test: Acidify a bit of feces with a few drops of glacial acetic acid and sprinkle benzidine powder mixed with sodium perborate over the resultant paste. The presence of a blue color indicates a positive reaction.

Guaiac test: Make the feces into a thick paste with water and acidify with a few drops of glacial acetic acid. To this add an equal volume of fresh alcoholic solution of gum guaiac. Mix and add an equal amount of hydrogen peroxide. A blue color indicates a positive reaction.

THE DETERMINATION OF HIPPURIC ACID IN URINE*

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THE estimation of hippuric acid in urine after the ingestion of benzoic acid was recommended by Quick¹ as a test for liver function. According to Weichselbaum and Probst² Quick's method proved to be unsatisfactory because of an incorrect solubility factor. They modified the method by saturating the urine with sodium chloride before precipitating it with acid and in this manner they not only diminished this solubility factor, but also caused it to be more constant. Employing this modification in our own laboratory, we were unable to obtain satisfactory results. We observed that different urines behaved differently and that the addition of more acid to the filtrates occasionally brought down a further precipitate. This suggested a critical evaluation of the optimum acidity for the quantitative precipitation of hippuric acid added to urine.

In both the original and the modified methods, it was apparent that the importance of pH was not sufficiently stressed. Thus, in the original publication Quick¹ states: "Acidify (acid to Congo red) with concentrated hydrochloric acid—about one c.c., stir vigorously." Weichselbaum and Probst² (page 637) state: "Add 1.2 c.c. of approximately 10 N sulfuric acid and scratch the sides of the flask with a rod to enhance crystallization, etc." Quick³ in a later publication (page 717) states: "Concentrated hydrochloric acid is added to make urine distinctly acid to Congo red or thymol blue—usually 1 c.c. of the acid is sufficient, but it is absolutely necessary to check with an indicator paper," and adds, "an excess of acid is permissible." It would be more exact if the latter statement were to read: *an excess of acid is imperative*. The following preliminary experiment bears out this point:

To 600 c.c. of normal urine acidified with acetic acid and brought to a boil to remove coagulable material, hippuric acid was added and the solution was filtered. Into each of four beakers 150 c.c. of this urine were measured. Forty-five grams of sodium chloride were added to each, then the procedure, as directed by Weichselbaum, was continued, varying the amount of 10 N sulfuric acid as follows:

	AMOUNT OF 10 N H ₂ SO ₄ ADDED (C.C.)	AMOUNT OF HIPPURIC ACID RECOVERED	
		(GM.)	(%)
1. Acid added as directed by Quick	1.0	1.20	70.6
2. Acid added as directed by Weichselbaum with very vigorous stirring	1.2	1.45	85.3
3. Same as 1; acid added until a precipitate formed and 0.5 c.c. excess	1.5	1.67	98.3
4. Same as 3 but twice the amount of acid was added	3.0	1.70	100.0

*From the Department of Biochemistry, Cook County Hospital, Chicago.
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These results show that the acidity used in beakers 1 and 2, as recommended by the previous authors, although definitely acid to Congo red and to thymol blue, is insufficient to precipitate the hippuric acid quantitatively. In beaker 4, where a large excess of acid was used, the precipitate did not redissolve.

TABLE I*

BEAKER NO.	HIPPURIC ACID (GM.)	SLIGHTLY ACIDIFIED URINE (C.C.)	10 N H ₂ SO ₄ ADDED (C.C.)	RECOVERY OF HIPPURIC ACID (GM.) (DUPLICATE)		% ERROR
1	0.5	100	3.2	0.505	0.51	+3.0
2	1.0	100	2.5	0.990	0.992	-0.1
3	1.5	100	1.2	1.510	1.470	-1.3
4	2.0	100	0.8	1.980	1.950	-2.0
5	3.5	100	0.0	2.640†	2.670†	-24.0†
			2.5	0.806	0.770	-1.7

*The solubility correction given by Weichselbaum was used.

†Precipitate formed on cooling to 20° C. without the addition of acid. To mother liquor 2.5 c.c. acid was added and an additional precipitate was obtained.

The next experiment was planned to simulate clinical conditions and to show the quantitative relationship between the amount of hippuric acid in urine and the amount of inorganic acid required to precipitate it quantitatively. Obviously, the extent of impairment of liver function for which the hippuric acid test is used clinically will vary with different patients, and consequently a varied amount of hippuric acid will be excreted. Since the normal output in a four-hour specimen is given as 3 to 3.5 Gm., it is reasonable to expect that clinically an excretion of from 0.0 to 3.5 Gm. or more can be expected. A normal slightly acidified urine was used as a base and a definite amount of purified crystalline hippuric acid (m.p. 185° to 187° C.) was weighed out into a series of beakers. Tenth normal sulfuric acid was added drop by drop until no more precipitate formed, then an additional 1 c.c. of acid was added. The results are tabulated in Table I. This table shows that the amount of 10 N sulfuric acid required to precipitate hippuric acid from 100 c.c. of urine quantitatively is inversely proportional to the amount of hippuric acid present. Thus, the less hippuric acid present, as in beaker 1, the more 10 N sulfuric acid necessary to precipitate it quantitatively. About 76 per cent of the hippuric acid in beaker 5 came down with no addition of inorganic acid; to get the remaining 24 per cent, 2.5 c.c. of 10 N sulfuric acid had to be added.

The estimation of hippuric acid was made by the titrimetric method rather than the gravimetric method recommended by Quick. The gravimetric procedure is out of the question when the precipitation is carried out in saturated salt solution. To rule out the objection raised by White and associates¹ that the precipitate in addition to hippuric acid might contain unconjugated benzoic acid and glucuronic acids and thus give erroneous acidity values by the titration method, the following experiment was made: The titrated hippuric acid solution was filtered quantitatively into a volumetric flask. The beaker and filter paper pulp were washed with distilled water into the flask until no test for chloride ion was obtained, and made up to volume. An aliquot portion was analyzed for total nitrogen and compared with the calculated nitrogen from the hippuric acid titration value. The results are shown in Table II.

TABLE II

SPECIMEN NO.	HIPPURIC ACID BY TITRATION (GM.)	CALCULATED NITROGEN (MG.)	MG. OF NITROGEN BY MICRO-KJELDAHL	% ERROR
1	3.75	293	300	+2.3
2	3.14	246	257	+4.4
3	2.85	223	237	+6.2
4	3.57	280	285	+1.7
5	4.12	322	343	+6.5
6	0.52	40.6	44	+8.3

These data warrant the conclusion that no other titratable acid is precipitated along with hippuric acid, and that the titration method is reliable.

The analytical procedure now used at the Cook County Hospital is as follows: Render the urine* specimen acid to litmus with acetic acid, bring to a boil to remove coagulable material, and filter if necessary. Measure the volume, and if it is more than 150 c.c., concentrate to 150 c.c. and add 30 Gm. of solid sodium chloride per 100 c.c. of urine, i.e., 45 Gm., and heat while stirring until dissolved. Cool to room temperature by immersion in cold water, and while stirring and scratching the sides of the beaker, add drop by drop 1:1 sulfuric acid until no more precipitate forms. (It is difficult to say how much acid to add as different urines require different amounts of acid. In general, it can be said that from 2 to 5 or 6 c.c. are usually sufficient. As a precaution we test each filtrate by adding more acid to see whether precipitation is complete.) Allow to stand for fifteen minutes or more and filter through a fluted filter paper. Wash the precipitate and beaker several times with a 30 per cent solution of sodium chloride (free of sulfate ion), using a fine tip wash bottle until the last washings give negative tests for sulfate ion. Transfer the precipitate with the filter paper back to the beaker in which the precipitation was carried out. Add about 75 or 100 c.c. of distilled water and heat until the precipitate dissolves. Titrate while hot, using 0.5 N sodium hydroxide and phenolphthalein as an indicator.

It was found convenient to report results in terms of sodium benzoate.

Calculations.—

$$1 \text{ c.c. of } 0.5 \text{ N NaOH} = 1 \text{ c.c. of } 0.5 \text{ N C}_7\text{H}_5\text{O}_2\text{Na}$$

$$1 \text{ c.c. of } 0.5 \text{ N C}_7\text{H}_5\text{O}_2\text{Na} = 0.072 \text{ Gm.}$$

$$\text{Therefore, } X \text{ c.c. of } 0.5 \text{ N NaOH} \times 0.072 \text{ Gm.} = \text{grams of C}_7\text{H}_5\text{O}_2\text{Na}$$

$$\text{Solubility correction for 150 c.c. of urine} = 0.15 \text{ Gm.}$$

SUMMARY

1. The acidity used by previous investigators was insufficient for quantitative precipitation of hippuric acid.
2. The amount of inorganic acid required for a quantitative recovery of hippuric acid varies with the composition of the urine and an excess of acid is desirable.

*If specimen is bile tinted, decolorize with norit by adding 0.3 Gm. per 100 c.c. or more, depending upon the amount of bile in specimen, and boil for about a minute, then filter by suction or fluted filter paper while hot.

3. It was shown that the titration method is reliable as no other organic acids are precipitated with the hippuric acid.

4. A modified quantitative procedure is given in detail.

REFERENCES

1. Quick, A. J.: The Synthesis of Hippuric Acid: A New Test of Liver Function, *Am. J. M. Sc.* 185: 630, 1933.
2. Weichselbaum, T. E., and Probst, J. G.: Determination of Hippuric Acid in Urine, *J. LAB. & CLIN. MED.* 24: 636, 1939.
3. Quick, A. J.: Intravenous Modification of the Hippuric Acid Test for Liver Function, *Am. J. Digest. Dis.* 6: 716, 1939.
4. White, F. W., Deutsch, E., Maddock, S., Downing, V., and Jensen, D.: The Comparative Value of Serial Hippuric Acid Excretion, Total Cholesterol, Cholesterol Ester, and Phospho-lipid Tests in Diseases of the Liver, *Am. J. Digest. Dis.* 6: 603, 1939.

THE DETERMINATION OF PHENOL RED WITH THE EVELYN COLORIMETER*

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THE use of phenol red to determine the dilution of test meals by the gastric secretion has been described by Hollander, Penner, and Saltzman.¹ Wilhelmj and Baea² modified this method by a correction for very dilute samples: mixing equal quantities of the sample and the original test meal before analysis. The purpose of this report is to record a further modification through the use of the Evelyn colorimeter³ for making the colorimetric comparisons.

To begin with, the test meal contains 1.0 mg. of phenol red per 100 c.c. instead of 2.0 mg. as used by both Hollander and Wilhelmj. When 5 c.c. of such a concentration are used as a standard, the galvanometer reading of the colorimeter is about 20. On the other hand, solutions containing as little as 0.1 mg. per 100 c.c. give a galvanometer reading of about 85. The Evelyn colorimeter thus gives a much wider working range than is feasible with the Duboseq colorimeter.

Our procedure is as follows: In a 76 by 120 mm. test tube, 5 c.c. of the sample are brought to a definite pink with finely powdered calcium oxide. Then 1 c.c. of 0.5 N sodium hydroxide and 2 c.c. of 0.3 N zinc sulfate are added and the tubes are shaken. After standing for ten minutes, the tubes are centrifuged. Five cubic centimeters of the clear supernatant liquid are placed in a colorimeter test tube and 5 c.c. of 0.5 N trisodium phosphate are added to develop the red color. The colorimeter tubes are then centrifuged for ten minutes at a speed of about 2,000 r.p.m. to throw down the zinc phosphate. The outside of the tube is wiped clean, and the reading is made using a water blank to adjust the galvanometer to 100. The filter used is Rubicon No. 565 because it transmits light of the wave length at which the alkaline form of phenol red has its maximum absorption.⁴

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This method eliminates one step entirely in the Hollander method, since the final reaction takes place in the colorimeter tube in which the reading is made. We have always obtained clear supernatants after centrifuging down the zinc phosphate. The volume of the precipitate is so small that its upper level remains below the light path from the colorimeter lamp.

When we first developed this technique, our studies were confined to water-glucose meals. At this time we prepared blanks by subjecting 5 c.c. samples of distilled water to our complete procedure. These blanks were used to set the galvanometer at 100 before taking our readings. We compared these settings with those obtained by using distilled water without preparation. Repeated tests showed the results to be identical, thus eliminating the need for a blank analysis. With Liebig's beef extract meals, blank analyses were made using the meal without phenol red. Although such solutions have a yellow color, with the filter of wave length 565 $m\mu$, they have a light absorption identical with that of water. We could thus eliminate the blank analyses again.

We have been using this technique for some time and find that the discrepancy between theoretical and observed values is about 2 per cent, or the equivalent of the experimental error of the Duboseq colorimeter. The use of a color filter transmitting light of a wave length which corresponds to the absorption maximum of phenol red in its alkaline form tends to eliminate interference from chromogens which were not removed by the precipitation with zinc sulfate and sodium hydroxide.

We have checked the relationship between the logarithm of the percentage light transmission and the concentration and have found that over the working range of the colorimeter it is linear. It should, therefore, be feasible to eliminate the standard and use a calibration curve. Since the stock solution of phenol red has a tendency to decrease in intensity,⁵ we believe the safer procedure is to prepare a standard from the test meal itself and thereby eliminate the possibility of error from such fading or other variation in making up the test meal. In addition to the fact that the use of the photoelectric colorimeter eliminates the obvious subjective factors involved in reading the Duboseq colorimeter, our method has the advantage of considerably shortening the time required to make the determinations.

SUMMARY

A modification of the Hollander method for determining phenol red applicable to the Evelyn colorimeter is described.

REFERENCES

1. Hollander, F., Penner, A., and Saltzman, M.: Determination of Phenol Red in Gastric Contents, *Proc. Soc. Exper. Biol. & Med.* 36: 568, 1937.
2. Wilhelmj, C. M., and Baca, D. E.: A Comparison of Methods for the Determination of Phenol Red in Gastric Contents, *J. LAB. & CLIN. MED.* 24: 207, 1938.
3. Evelyn, K. A.: A Stabilized Photoelectric Colorimeter With Light Filters, *J. Biol. Chem.* 115: 63, 1936.
4. Evelyn, K. A.: Evelyn Photoelectric Colorimeter Notes on Operation, Philadelphia, 1939, Rubicon Co.
5. Schoonover, Janetta W., and Woodward, G. E.: Some Refinements Upon the Colorimetric Method of Hastings and Sendroy for the Determination of pH of the Blood, *J. LAB. & CLIN. MED.* 16: 621, 1931.

STUDIES ON THE METABOLISM OF IRON AND COPPER*

I. METHOD FOR THE DETERMINATION OF IRON AND COPPER IN BLOOD SERUM

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AGNES SCHMIT, M.S., OMAHA, NEB.

EXPERIMENTS have been conducted in this laboratory for the past seven years to obtain data on the metabolism of iron and copper in the human body. We established the normal values for whole blood iron and whole blood copper for persons of various ages and of both sexes.¹⁻⁴ We also reported whole blood iron and copper values in the various anemias and other pathologic conditions.^{2, 5}

We found that a reciprocal relationship existed between the iron and copper content of the whole blood in anemias and allied pathologic conditions.^{2, 5} When the whole blood iron was decreased, the whole blood copper increased; and likewise when the whole blood iron increased, the whole blood copper decreased. Hypereupremia was the usual response to hypoferronemia.

Recent investigations suggest that the blood serum serves as the medium for transportation of iron in the body.⁶⁻⁸ Since copper is so closely associated with iron in the hematopoietic process, it is logical to believe that it also may be transported in the serum. Our present study of the iron and copper content of the blood serum was undertaken to gather data relative to the absorption and utilization of these two important biological elements.

The serum normally contains very small quantities of copper and non-hemoglobinous iron. Our first effort then was to establish a satisfactory method for the determination of serum iron and copper. Repeated efforts to determine the iron and copper content in the same sample of serum failed to give reliable results, hence a method was devised for the analysis of aliquot portions of serum separately for iron and copper. The chief difficulty in the latter method is that serum iron in the presence of the copper reagent, sodium diethyl-dithiocarbamate, produces a brownish color which interferes with the yellow color used for the detection of copper. Other workers also have observed this difficulty.⁹ Our attempts to overcome this interference by binding the iron with sodium pyrophosphate were successful. The binding effect of sodium pyrophosphate is demonstrated in Table I, where solutions containing known amounts of iron and copper were treated with saturated sodium pyrophosphate, ammonium hydroxide, and sodium diethyl-dithiocarbamate, respectively, and the color extracted with isoamyl alcohol. The colored alcohol layers were transferred to microcolorimeter cups and compared in the Klett colorimeter, using solution I as the standard.

*From the Departments of Medicine and Biological Chemistry, Creighton University School of Medicine, Omaha.

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Obviously since solutions I and VIII (Table I) checked with one another, no contamination was introduced through the use of sodium pyrophosphate. Furthermore, in solutions II, III, and IV (Table I), the experimental values agreed with the actual values, while in solutions V, VI, and VII (Table I) they did not. This fact led us to believe that the binding of serum iron is essential in the sodium diethyl-dithiocarbamate method for the determination of serum copper.

TABLE I

EFFICACY OF SODIUM PYROPHOSPHATE TO BIND SERUM IRON

SOLUTION NO.	FE AND CU PRESENT (MG.)	Na ₂ P ₂ O ₇ ·10H ₂ O* (C.C.)	NH ₄ OH (C.C.)	CARBAMATE (C.C.)	ALCOHOL (C.C.)	COLORIMETER READING (MM.)	COPPER		% ERROR
							EXP. (MG.)	ACTUAL (MG.)	
I	0.010 Cu 0.000 Fe	1	2	2	4	15.00	--	0.010	--
II	0.010 Cu 0.020 Fe	1	2	2	4	15.00	0.010	0.010	0
III	0.010 Cu 0.010 Fe	1	2	2	4	15.00	0.010	0.010	0
IV	0.005 Cu 0.010 Fe	1	2	2	4	30.00	0.005	0.005	0
V	0.010 Cu 0.020 Fe	0	2	2	4	17.60	0.008	0.010	20
VI	0.010 Cu 0.010 Fe	0	2	2	4	15.80	0.009	0.010	10
VII	0.005 Cu 0.010 Fe	0	2	2	4	33.60	0.004	0.005	10
VIII	0.010 Cu 0.000 Fe	0	2	2	4	15.00	0.010	0.010	0

*Redistilled water, free from copper and iron, was used to prepare the sodium pyrophosphate solution.

From the results in this table we concluded that 1 c.c. of a saturated sodium pyrophosphate solution should bind completely the interfering iron present in the samples of serum used for our copper analyses, since our normal serum iron values rarely exceeded 0.020 mg. of iron per 5 c.c. of serum.

PROCEDURE

Reagents and Apparatus.—All reagents and apparatus used in these experiments were rendered free of iron and copper. Acids and water were redistilled from all-glass distillation apparatus until free from iron and copper.* The ammonium hydroxide (28 per cent) was found to be copper free, and consequently it was unnecessary to purify it.† Blanks were run frequently to check the absolute purity of the reagents used. The syringes and platinum needles used for drawing the blood samples were carefully rinsed with redistilled water before sterilization by autoclaving.

Using a sterile syringe, 37 c.c. of blood were drawn between 8:00 A.M. and 9:00 A.M. from donors who had fasted for at least twelve hours. Two 14 c.c. samples were transferred immediately to clean, dry centrifuge tubes and centrifuged. In the transfer of the blood from the syringe to the tubes, the blood was allowed to run slowly down the side of the tube without any external

*Qualitative test for iron: 5 to 10 c.c. of solution to be tested are treated with 1 c.c. of HCl, 0.1 c.c. of concentrated HNO₃, 4 c.c. of 3 N KCNS, and 2 c.c. of isoamyl alcohol. If the alcohol layer is colorless, the test solution is free from iron.

†Qualitative test for copper: 5 to 10 c.c. of solution to be tested are treated with 0.5 c.c. of 28 per cent NH₄OH, 2 c.c. of sodium diethyl-dithiocarbamate, and 2 c.c. of isoamyl alcohol. If the alcohol layer is colorless, the test solution is free from copper.

†The ammonium hydroxide was obtained from the Mallinckrodt Chemical Co.

pressure on the plunger. Likewise when the blood was withdrawn from the veins, no suction was exerted on the plunger. This precaution must be taken in order to avoid hemolysis. The remaining blood was transferred to a large test tube containing 15 mg. of sodium oxalate and was used subsequently for the determination of hemoglobin and of whole blood iron and copper, cell volume, and for the making of red and white blood cell counts. The samples from which the serum was obtained were centrifugated at 1,500 r.p.m. for one and one-half to two hours. After centrifugation the serum was carefully removed from the cells and tested for hemolysis.

Testing the Serum for Hemolysis.—Using a 5 c.c. Ostwald-Folin pipette, a sample of serum slightly in excess of 5 c.c. was withdrawn, and the serum allowed to flow into a dry receptacle until the 5 c.c. mark was reached. This serum was employed for the benzidine test. The remaining 5 c.c. were transferred quantitatively to a vitreosil dish.

Benzidine Test for Hemolysis.—To a clean 15 c.c. test tube exactly two drops (equivalent to 0.2 c.c.) of the blood serum for the benzidine test, 1 c.c. of 1 per cent benzidine dihydrochloride* solution, and 1 c.c. of 3 per cent hydrogen peroxide were added. The 3 per cent hydrogen peroxide was prepared from superoxol (30 per cent H_2O_2) by dilution. If a blue color appeared, the benzidine test was positive, indicating the presence of hemoglobin. A serum sample giving a positive benzidine test was not used for serum iron determinations. It was used for serum copper determinations, however, if only a slight blue color was obtained in the benzidine test. Our results have indicated that traces of hemoglobin do not affect the accuracy of serum copper analyses.

We have found this benzidine test capable of detecting hemoglobin in blood which has been diluted 1:100,000; that is, 1 c.c. of this diluted sample containing 1.14 μg of hemoglobin, or 0.0038 μg of hemoglobin iron, gave a positive benzidine reaction. The hemoglobin content of the blood sample was calculated from the iron content of 5 c.c. of whole blood, as determined by the dry ash method. This test was performed on each freshly prepared solution of benzidine dihydrochloride to insure the constant sensitivity of our benzidine test.

Because this test is capable of detecting 0.0038 μg of hemoglobin iron, a negative benzidine reaction in 0.2 c.c. of serum would indicate that there is no more than 1.9 μg of hemoglobin iron in 100 c.c. of serum. This amount of iron falls well within the limits of experimental error, and, therefore, may be considered negligible.

SERUM IRON DETERMINATION

Five cubic centimeters of benzidine negative serum were transferred to a vitreosil dish and evaporated to dryness on the hot plate, with constant stirring to prevent spattering. The contents were covered and ashed for eight hours in an electric furnace. One cubic centimeter of concentrated nitric acid was then added, and the contents were evaporated to dryness on the hot plate. If ashing was incomplete, it was finished at this stage by heating gently over an open flame until all traces of carbon disappeared. Two and one-half cubic centimeters of 6 N hydrochloric acid were added, and the solution was warmed slightly to dissolve the ash completely. The contents were transferred quantitatively to a

*The crystalline benzidine dihydrochloride was obtained from Eastman Kodak Co. A 1 per cent solution of the salt was stable for at least a month.

50 c.c. glass-stoppered cylinder, the dish was washed carefully with redistilled water containing 0.5 c.c. of 6 N hydrochloric acid, and the wash was transferred to the cylinder.

The standard was prepared as follows: Using a 1 c.c. Ostwald-Folin pipette and a standard iron solution containing 10 μg of iron per cubic centimeter, 1 c.c. of the iron solution (equivalent to 10 μg of iron) was transferred to a 50 c.c. glass-stoppered cylinder, and 3 c.c. of 6 N hydrochloric acid were added.

To both standard and unknown 0.1 c.c. of concentrated nitric acid was added, and the containers were rotated gently to insure thorough mixing. Exactly 4 c.c. of 3 N potassium thiocyanate and 4 c.c. of isoamyl alcohol were added. The cylinders were stoppered and shaken for one to two minutes and then allowed to stand until the alcohol layer separated from the aqueous solution.

Using a small pipette, the colored alcohol layer was transferred to a dry acid-washed filter paper, 1 inch in diameter, and filtered into a microcolorimeter cup. Comparison in the colorimeter was made as soon as possible.

SERUM COPPER DETERMINATION

Five cubic centimeters of serum were transferred to a vitreosil dish and evaporated to dryness, with constant stirring to prevent spattering. The contents were covered and ashed for eight hours in an electric furnace. One cubic centimeter of concentrated nitric acid was added, and the contents were evaporated to dryness on the hot plate. To insure complete ashing the residue was heated gently over an open flame until all traces of carbon disappeared. The ash was then dissolved in 2.5 c.c. of 6 N hydrochloric acid, warming slightly to dissolve it completely, and transferred quantitatively to a 50 c.c. glass-stoppered cylinder. The dish was finally washed with redistilled water containing 0.5 c.c. of 6 N hydrochloric acid, and the washings were transferred directly to the cylinder.

A copper standard was prepared as follows: Using a 1 c.c. Ostwald-Folin pipette and a standard copper solution containing 10 μg of copper per cubic centimeter, 1 c.c. of the copper solution was transferred to a 50 c.c. glass-stoppered cylinder, and 3.0 c.c. of 6 N hydrochloric acid were added.

Using redistilled water, both standard and unknown were made up to a volume of approximately 35 c.c. To both standard and unknown 2 c.c. of concentrated ammonium hydroxide were added. The solutions were cooled, and 1 c.c. of a saturated solution of pyrophosphate, 2 c.c. of an aqueous solution of 0.5 per cent sodium diethyl-dithiocarbamate reagent, and 4 c.c. of isoamyl alcohol were added. The contents were shaken for one to two minutes and then allowed to stand until the alcohol layer separated from the aqueous solution. Using a small pipette, the colored alcohol layer was transferred to a dry acid washed filter paper as for serum iron, and filtered into a microcolorimeter cup. Comparison was made in the colorimeter.

Some of the serum iron and copper values which we have obtained using the method outlined are listed in Table II. These samples were obtained from normal male college students, 20 to 30 years old.

TABLE II

WHOLE BLOOD AND SERUM IRON AND COPPER CONTENT OF MALE ADULTS

NAME	RED BLOOD CELLS	WHITE BLOOD CELLS	HEMO- GLOBIN* (GM./ 100 C.C. BLOOD)	SERUM		WHOLE BLOOD		IRON COLOR INDEX†	CELL VOL. %
				FE MG./100 C.C. SERUM	CU MG./100 C.C. SERUM	FE (MG./ 100 C.C. BLOOD)	CU (MG./ 100 C.C. BLOOD)		
Kok	4,384,000	6,500	15.21	0.127	0.114	50.96	0.127	1.18	48.5
Her	4,768,000	13,900	14.35	0.112	0.092	49.08	0.130	1.04	45.8
Cah	4,672,000	5,550	14.35	0.117	0.091	49.08	0.115	1.06	46.0
Gre	4,928,000	7,900	13.19	0.170	0.115	44.20	0.120	0.90	48.0
Cow	4,736,000	5,400	16.02	0.135	0.091	53.68	0.123	1.14	51.2
Rus	5,376,000	5,850	16.23	0.168	0.132	54.37	0.135	1.02	51.2
Mak	4,992,000	5,700	15.16	0.140	0.094	50.80	0.120	1.03	49.1
Sto	4,864,000	12,100	15.91	0.258	0.084	53.32	0.140	1.11	48.0
O'L	5,280,000	7,800	17.19	0.103	0.129	57.56	0.118	1.10	--
He	4,896,000	8,400	14.95	0.170	0.108	50.10	0.121	1.04	46.8
Average			15.25	0.150	0.105	51.30	0.125	1.06	

*The hemoglobin values were calculated from the whole blood iron values, using the Butterfield factor:

$$\text{Hemoglobin, Gm. in 100 c.c. of blood} = \frac{\text{Milligrams of iron in 100 c.c. of blood}}{3.35}$$

$$\text{†Iron color index} = \frac{\text{Milligrams of iron in 100 c.c. of blood}}{\text{First two figures of red blood cell count}}$$

COMMENT

On the whole, the serum iron values which we have obtained using the method outlined (0.103 to 0.258 mg. per 100 c.c. of serum) fall within the range reported by other investigators who used different methods. Moore and his collaborators, from an analysis of fifteen male subjects, reported a range of 0.094 to 0.174 mg. per 100 c.c.⁶ Walker¹⁰ reported a range of 0.040 to 0.230 mg. per 100 c.c. from a series of twelve medical students. Fowweather¹¹ obtained a range of 0.095 to 0.180 mg. per 100 c.c. Other workers, not specifying the sex studied, reported such values as the following for 100 c.c. of serum: Locke, Main, and Rosbash, 0.083 to 0.115 mg.; Warburg and Krebs,¹² 0.067 to 0.116 mg.; Barkan,¹³ 0.056 to 0.140 mg.; and Langer,¹⁴ 0.050 to 0.180 mg.

We have observed in an occasional case a marked deviation from the general range of values reported. This suggests the possibility that the absorptive state of the individual and the iron content of his diet for a period of time preceding the analysis are possible factors affecting serum iron concentrations.

The serum copper values we have obtained (0.082 to 0.132 mg. per 100 c.c. of serum) are slightly higher than those of Locke, Main, and Rosbash,⁹ who reported a range of 0.073 to 0.087 mg. per 100 c.c. for eight cases, and of Warburg and Krebs,¹² who stated 0.082 mg. per 100 c.c. as their average value for ten adults.

We are continuing our determinations so as to establish a larger series of normals that may give us a more accurate range of normal values which we intend to publish at a later date.

SUMMARY

1. A relatively simple dry ash method has been described for the determination of iron and copper in blood serum. Aliquot samples of serum were evaporated to dryness and ashed in an electric furnace for eight hours; the ash was

treated with nitric acid, dissolved in dilute hydrochloric acid, and transferred to glass-stoppered cylinders. The solution in one cylinder was then analyzed for serum iron, the other for serum copper. For iron analysis the solution was treated with nitric acid, 3 N potassium thiocyanate solution, the color was extracted with isoamyl alcohol, and the unknown was compared with a standard in a colorimeter. For copper analysis the solution was treated with ammonium hydroxide, sodium pyrophosphate, and sodium diethyl-dithiocarbamate. The color was extracted with isoamyl alcohol and the unknown was compared with a standard in the colorimeter.

2. Using this method, values for the iron and copper content of the serum of ten male adults are reported. The serum iron values range from 0.103 to 0.258 mg., giving an average of 0.150 mg. per 100 c.c. of serum. The serum copper values range from 0.084 to 0.132 mg., yielding an average of 0.105 mg. per 100 c.c. of serum.

REFERENCES

1. Sachs, A., Levine, V. E., and Appelsis, A.: Iron in Human Blood, *Arch. Int. Med.* 52: 366, 1933.
2. Sachs, A., Levine, V. E., and Fabian, A.: Copper and Iron in Human Blood, *Arch. Int. Med.* 55: 227, 1935.
3. Sachs, A., Levine, V. E., and Fabian, A.: Copper and Iron in Human Blood, *Arch. Int. Med.* 58: 523, 1936.
4. Sachs, A., Levine, V. E., and Griffith, W. O.: Copper and Iron in Human Blood, *Arch. Int. Med.* 60: 932, 1937.
5. Sachs, A., Levine, V. E., and Griffith, W. O.: Reciprocal Relationship of Copper and Iron in Blood. Polycythemia Vera, *Proc. Soc. Exper. Biol. & Med.* 35: 6, 1936.
6. Moore, C. V.: Studies in Iron Transportation and Metabolism. I. Chemical Methods and Normal Values for Plasma Iron and "Easily Split-off" Blood Iron, *J. Clin. Investigation* 16: 613, 1937.
7. Moore, C. V., Minnich, V., and Welch, J.: Studies in Iron Transportation and Metabolism. III. The Normal Fluctuations of Serum and "Easily Split-off" Blood Iron in Individual Subjects, *J. Clin. Investigation* 18: 543, 1939.
8. Moore, C. V., Arrowsmith, W. R., Welch, J., and Minnich, V.: Studies in Iron Transportation and Metabolism. IV. Observations on the Absorption of Iron From the Gastro-Intestinal Tract, *J. Clin. Investigation* 18: 553, 1939.
9. Locke, A., Main, E. R., and Rosbash, D. O.: The Copper and Iron Contents of the Blood Serum in Disease, *J. Clin. Invest.* 38.
10. Walker, B. S.: The Iron of Human Blood Serum, *J. Lab. Clin. Med.* 38.
11. Fowweather, F. S.: The Determination of Iron in Blood Plasma, *Biochem. J.* 28: 1160, 1934.
12. Warburg, O., and Krebs, H. A.: Über locker gebundenes Kupfer und Eisen im Blutserum, *Biochem. Ztschr.* 190: 143, 1927.
13. Barkan, G.: Über Bestimmungsmethodik und Eigenschaften des "leicht abspaltbaren" Bluteisens, *Ztschr. f. physiol. Chem.* 216: 1, 1933.
14. Langer, A.: Über den Gehalt an Ferrieisen in normalen und pathologischen Seren, *Biochem. Ztschr.* 242: 316, 1931.

MEDICAL ILLUSTRATION

AN INTRODUCTION TO ILLUSTRATION

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WITH ILLUSTRATIONS BY ELIZABETH S. CONE AND CARL DAME CLARKE

IN ESTABLISHING the Department of Medical Illustration in this JOURNAL I feel that an introduction to illustration is in order. This subject has been discussed somewhat at length in previous articles and books.¹⁻⁴ At best this introduction is only a short summary to call to the reader's attention the different types of illustration and how they may be used.

For the purpose of portrayal the scientist has at his disposal three mediums of illustration. The first of these is photography, the second is drawing or painting, and the third is modeling. In this latter medium, molds and casts of the actual subject are employed more than sculptured models. For the purposes of medicine photography is used more than drawing, and drawing is used more than modeling. It is common for the scientist to overlook the possibilities of one medium of illustration in his enthusiasm for another.

There are some cases in which the photograph will serve as a better illustration than the drawing, and other cases in which the drawing is indispensable. The wax or rubber model serves best in the small classroom where it can be used during a didactic lecture. *The medium of illustration to be chosen depends entirely on the subject to be illustrated and the purpose for which the illustration is to be used.* The photograph is the cheaper method and takes less time to produce. Naturally, it is the most popular method of illustration. There are cases in which it is absolutely impossible for the photograph to show to good advantage in comparison with the drawing. There are also instances in which the making of a drawing would be an unnecessary expense and a waste of time when the photograph would be sufficient.

Examples of cases where the ordinary photograph is suitable are as follows: skin lesions in which color is of little importance; deformities of any part of the body that manifest themselves on the outer surfaces; pathologic specimens void of color, as after fixation in formalin; apparatus of various types, such as chemical, surgical, and medical. In many cases pen-and-ink diagrammatic line drawings of apparatus show to better advantage the operation of such equipment.

There are cases in which the best photograph is worthless in comparison with the drawing made by the finished artist. It is not only hazardous, but practically impossible, to obtain a photograph through the small incision that is made

to gain access to the abdominal and thoracic viscera. Even the most proficient photographer, using the best equipment, is likely to fail or refuse to try. Without doubt the mechanics of surgery are best illustrated through the medium of draftsmanship. Even the simple pen-and-ink diagram is often superior to the most perfected photograph in presenting a surgical procedure for mental absorption.

In many cases drawings are superior not only because of the inaccessibility of the field, but also because important structures are often covered by blood, mucus, fascia, fat, and other unimportant parts that prevent a clear view to the lens of the camera. The artist can eliminate these obstructions from his drawing, but it is generally impossible for the photographer to do so in his photograph. Of course, photographs can be retouched, but when any great amount of retouching is done the photograph becomes more of an art job than a photograph. To this end it would have been better for the artist to have made the entire illustration.

In comparing the drawing and the photograph made through the microscope, it often becomes a matter of personal taste on the part of the physician as to his preference. I am of the opinion that the average physician is more accustomed to poor photomicrographs than he is to good ones. Not all physicians are fortunate enough to have at their disposal trained medical artists and photographers. In fact, the making of medical pictures is often left to artists and photographers doing the usual run of commercial work. Few, if any, commercial photographers have the equipment for making photomicrographs, and since this is a highly developed and specialized field of photography, few of them understand how to obtain the best results. Therefore, photography of this type is left to the pathologist or laboratory worker, who may be very proficient in his own field but a poor photographer. He may consider his results good until a comparison with truly excellent photographs is made.^{6, 7}

The photomicrograph of a few years ago was a poor excuse for a good drawing made at the same time of the same subject. With the continued development and use of panchromatic photographic plates, color filters, apochromatic lenses, and perfected photomicrographic apparatus, the drawing of the microscopic subject is beginning to recede into the background. Photomicrographs of any microscopic section can be made through any known and used power of lenses. In other words, low-power pictures covering the entire section can be produced as well as the pictures made through the oil-immersion lens. Any degree of magnification between these two can be successfully obtained and the section photographed at that specified power. Magnifications between those given by combinations of specified oculars and objectives may be obtained by extending the bellows of the photomicrographic camera.

It is possible for the artist to accentuate any part of the cell structure as well as "hold back" the insignificant parts in a microscopic slide. The photographer can do the same thing by the use of the proper color filter or filters, with the correct plate and slight variations in exposure, and development of the negative and positive. Small sections of a negative may be accentu-

ated by the use of a dye on other parts of the negative to hold them back during the printing, or by the use of tissue paper beneath the negative in the printer. It stands to reason that the photograph is a truer representation of the original. However, in high-power work detail and depth in a cell are often difficult to obtain by photography. The artist can improve on some photomicrographs because he can make a composite picture of what comes in and out of view as he focuses his microscope up and down. A high-power photomicrograph may be good as far as the photographic technique is concerned, but a drawing of the same subject can be superior because of the ability of the eye and hand to record on paper in a single picture the various depths of a microscopic section.



Fig. 1.—A typical half-tone drawing for the illustration of the mechanics of surgery.

It is possible to combine drawing with photography to produce a drawing or a picture composed of both drawing and photography. The silver print or photochemical method of combining drawing and photography is a procedure that can be applied to the making of any type of drawing. However, it is more suitable for pen-and-ink illustrations. It can be used for the wash, water color oil color, tempera, and even pencil dust techniques. This subject has already been discussed fully⁸ and will not be dealt with in this introduction.

Natural color photographs can be made and used for lantern slides or publication. These pictures in color are made on glass or celluloid film and are often exceptionally well done. As a rule, the natural color photograph compares favorably with the best color drawings used in scientific publications. However, the artist has at his disposal many colors from which he may make hundreds of combinations. The natural color photograph is generally composed of three

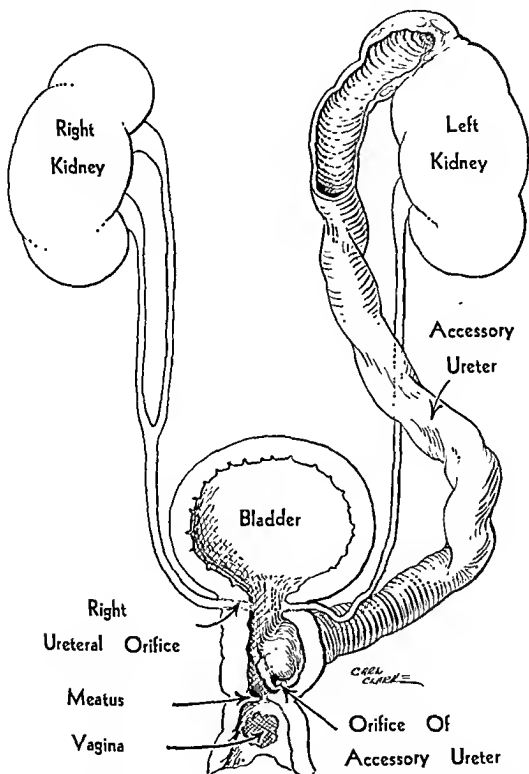


Fig. 2.—Sometimes a simple pen-and-ink semidiagrammatic sketch serves best as a drawn illustration.

colors; therefore all colors must be those that are originally in the emulsion or the combinations that can be obtained by optically mixing these colors. A good color photographer will invariably produce a better color picture than a poor artist and, in turn, a good artist will produce a better color picture than a poor photographer.

The artist may be able to color or tint photographs, but the drawing or painting by the skilled artist without photographic aid is usually superior. Photographs to be colored or tinted as medical illustrations should be made first in sepia tone. This is necessary as the black undertone has a tendency to "kill" or destroy the brilliancy and strength of warm colors such as reds and yellows. These colors are used in painting or glazing over the photograph.⁹

One of the important factors to be considered before having full-tone colored pictures made, whether colored photographs or paintings, is the expense of reproduction. This expense is augmented by the necessity of making numerous photoengraved cuts, one of each color, and the added processes of printing. Many medical journals require the author, or the institution with which he may be affiliated, to bear the cost of color reproductions. However, the cost of full-tone color pictures is greatly reduced by using flat color only. Structures such as veins, nerves, and arteries, can be accentuated by the use of flat color over black-and-white half tones or line drawings. The color illustrations in the *Hand Atlas of Human Anatomy* by Spalteholz are examples of the use of flat color in photoengraving. The average magazine cover is generally reproduced in full tone.

It is my assumption that better reproductions in black and white are obtained from photographs or drawings that are themselves in black and white rather than in color. For this reason, if the picture is not to be reproduced in color, the artist or photographer should make the original in black and white.¹⁰

Practically all lantern slides are made by the photographic process, regardless of what the original may be. One exception to this rule is when the diagram or sketch is drawn directly on a prepared glass of lantern slide size; another is the process of typewriting through carbon paper placed next to a piece of cellophane or celluloid. This latter method gives a carbon impression on the cellophane, which is then mounted between two lantern slide cover glasses for projection.¹¹

The same problems, as well as many others that arise in still photography, apply to motion picture photography. The scientist should write out a scenario before proceeding. This is of considerable importance in making excellent professional motion pictures. Generally a picture is taken of a surgical operation or similar procedure. After the film is developed, titles are inserted or the film is scored with sound. Few scientific films tell their stories as clearly or coherently as a logical sequence of events.¹²

The scientist should bear in mind that the preparation of a motion picture is comparable to the preparation of an article for publication. An outline or scenario is made, the material is gathered and then photographed, the negative is edited, and finally the negative is titled or scored with sound and printed. This produces a continuous film without patches which cause noise and shaking on the screen. Most persons prefer to edit a positive print, then make the negative correspond to the positive. However, if the editing is done on the negative, the expense of an additional positive is eliminated. A scientific subject that is worth recording is usually worth making into both the negative and the positive rather than reversing the negative into the positive, which is the

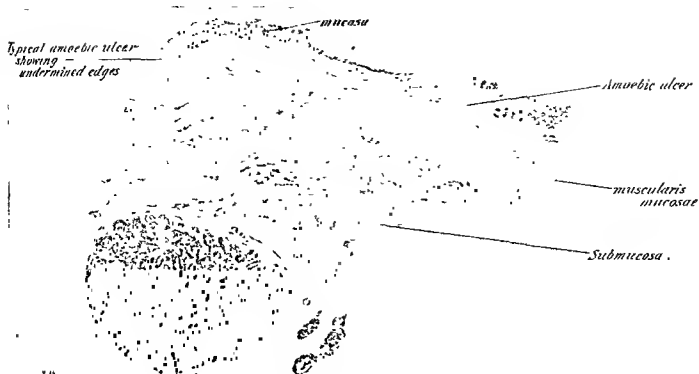


Fig. 3.—In the past most microscopic subjects were drawn, as is shown in this illustration.

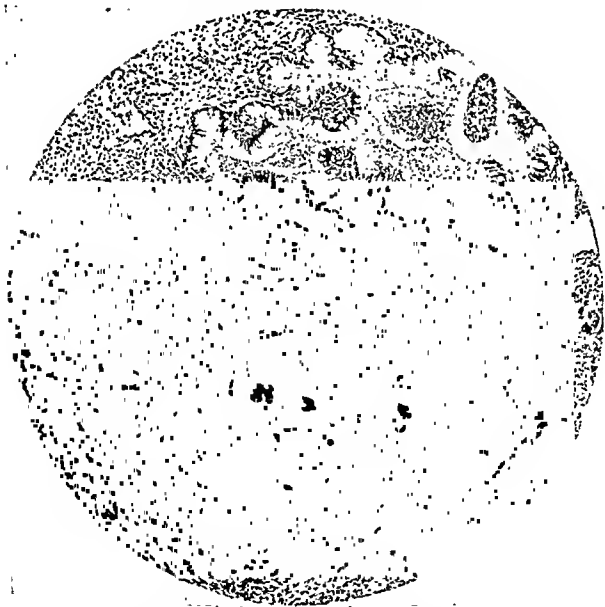


Fig. 4.—The above photomicrograph of a case of tuberculosis of the cervix may be used for comparing with the drawn microscopic picture in Fig. 3.

general rule. It is slightly more difficult to follow the action on the negative than on the positive. The difference in price between Kodachrome or color film and panchromatic film warrants the use of color film entirely for most scientific subjects. This positive color film should be edited, titled, or scored with sound, and immediately duplicated before it has been run long enough to receive annoying scratches.

Animated drawings are playing an important part in scientific illustration. Their production is so highly technical that it becomes a specialized field within itself. The artist who intends to make acceptable animated drawings should, in addition to his knowledge of art, understand the fundamentals of photography, especially motion picture photography. These fundamentals embrace lens action, such as focusing, focal length, and speed of lenses, as well as exposure; the mechanics of the time lapse motion picture camera; and, finally, the technique of the processing room, for it is here that many interesting and necessary animated effects are produced with chemicals and the printing machine.

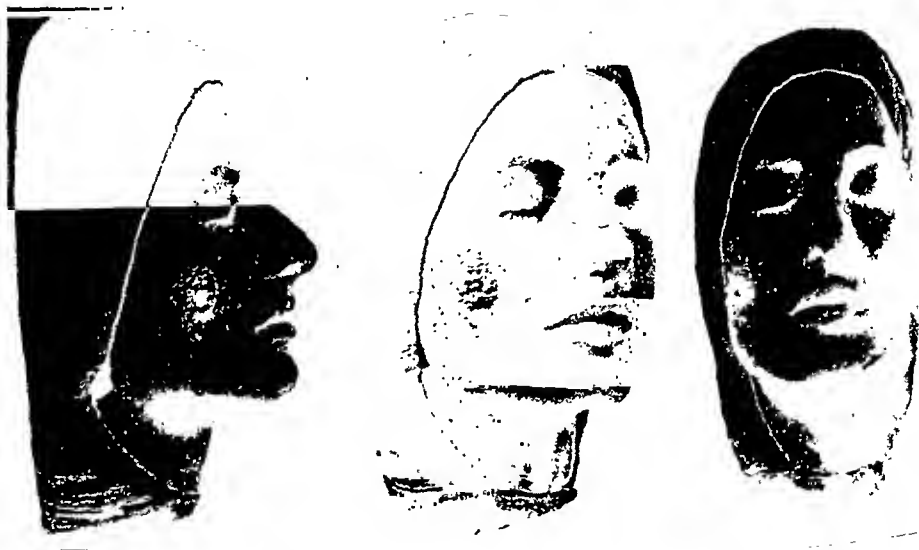


Fig. 5.—The three-dimensional wax cast serves best for museum purposes and for study in the small classroom.

These, of course, apply only to the artist who does his own camera work. However, it is advantageous to learn the fundamentals of the entire process. Photography and drawing are so interdependent for the animation worker that a description of one is inadequate without a description of the other. The motion picture, when properly conceived, photographed, and edited, becomes man's most powerful tool for the dissemination of knowledge. Animation provides a means of obtaining motion pictures which cannot be produced by ordinary cinematography. In other words, animated drawings accomplish the same purpose for the motion picture that an ordinary drawing accomplishes for the textbook. It seems evident that animated drawings will play an even greater part in the dissemination of scientific knowledge than they do at present. This is indeed a fascinating branch of scientific illustration and will not only be in-

teresting to the artist and photographer, but will also tax his ingenuity and imagination in developing new processes and equipment.¹³

Wax or plaster casts can be made of skin lesions, deformities, and abnormal growths and depressions of the body. These molds and casts are made by first applying liquid plaster of Paris or an agar composition to the properly prepared surface. When these materials have set and are removed, they constitute the mold, which in turn is filled with plaster, wax, celluloid, or rubber. The mold is removed after the hardening of the positive material, thereby producing an exact impression which shows such details as the pores of the skin, provided the work has been done properly.

The agar composition is pliable enough to permit free removal of the mold from the anatomic part in cases where there is a degree of undercutting. The agar composition¹⁴ is more suitable for use on the living body or on fresh structures after removal from the body. For the inanimate object composed of plaster or wax the rubber mold serves best. The simplest rubber molds are made from prevulcanized latex combined with cellulocotton.

By the use of molds and casts lifelike reproductions can be made of extremities, skin conditions, or any part of the abdominal or thoracic viscera after its removal from the body. These casts can be colored to represent the originals and often have been mistaken for the actual organ or part.

Cut or destroyed parts of specimens can be reconstructed before casting. If the worker is clever, he can model or sculpt the entire subject in clay, wax, or plasteline. From his original work a rubber or plaster piece mold can be made to reproduce a number of casts in colored wax or plaster.⁴

Casts are considered to be the best representation of the original but are more cumbersome to handle in exhibiting than the photograph or drawing. Photographs and lantern slides can be made from them for reproduction or projection. Wax or plaster casts serve best as museum pieces. They can also be used for student instruction in small groups.

The possibilities of moulage making are unlimited. Its value to the human race warrants further study and rapid propagation. As an example, consideration should be given to the crude, artificial anatomic appliances which are attached to the body in the vain hope that they will make the unfortunate wearer happier, more presentable to society, and better equipped to cope with the ever-mounting competition of commercial, social, and economic existence. It is indeed deplorable that such prostheses have not been perfected to a degree that would produce realistic, natural, and comfortable appliances of practical use. The present-day artificial limbs are examples of prostheses that lack realism. False teeth have been perfected to a greater extent. There is little reason why other artificial parts should not be developed to the same degree.^{4, 14, 15}

An effort should be made to establish a technique in its most simple form. When this is accomplished, other incomplete fields may be conquered and additional knowledge may be gained. In fact, new ideas will develop and encourage the worker to return to the original problem in order to raise it one step nearer the unattainable peak of perfection. The goal is unlimited but the zest of trying is worth the effort.

In conclusion, it may be repeated that the subject to be depicted and the purpose for which the illustration or reproduction is to be used determine the medium through which it should be produced. It will be well worth the physician's time to discuss this matter fully with the artist and the photographer before he blindly orders a paper to be illustrated. It is hoped that such a discussion will improve medical illustrations and their worth in conveying ideas from one person to another.

REFERENCES

1. Clarke, C. D.: Pictorial and Plastic Medical Illustrations, J. LAB. & CLIN. MED. 18: 1165, 1933.
2. Clarke, C. D.: Illustration, Its Technique and Application to the Sciences, Baltimore, 1940, John D. Lucas Co.
3. Clarke, C. D.: Photographic Methods in Medical Illustration, Their Applications, Limitations and Substitutes, J. Tech. Methods 19: 27, 1939.
4. Clarke, C. D.: Molding and Casting, Its Technique and Application, Baltimore, 1933, John D. Lucas Co.
5. Clarke, C. D.: Apparatus for Macrophotography, J. Biol. Photographic Assn. 2: 76, 1932.
6. Photomicrography, ed. 13, Rochester, 1935, Eastman Kodak Co.
7. Gage, Simon Henry: The Microscope, Ithaca, N. Y., 1932, Comstock Publishing Co.
8. Clarke, C. D.: Combining Drawing and Photography for Composite Illustration, J. Biol. Photographic Assn. 3: 37, 1934.
9. The Photography of Colored Objects, ed. 12, Rochester, 1933, Eastman Kodak Co.
10. The Art of Photoengraving, American Photoengravers Assn., 1929.
11. Lantern Slides: How to Make and Color Them, Rochester, 1927, Eastman Kodak Co.
12. Clarke, C. D.: The Movies in Medicine, Hygiea 8: 509, 1930.
13. Clarke, C. D.: The Fundamentals of Animated Scientific Drawing and Cinematography, J. Biol. Photographic Assn. 6: 1, 1937.
14. Bulbulian, A. H.: An Improved Technique for Prosthetic Restoration of Facial Defects by Use of a Latex Compound, Proc. Staff Meet., Mayo Clin. 14: 433, 1939.
15. Bulbulian, A. H.: Prosthetic Restorations of Facial Defects by Use of Latex Compound: A Further Detailed Description of the Technique Used, Proc. Staff Meet., Mayo Clin. 14: 721, 1939.

Erratum

On page 554 of the December number of the JOURNAL in the article by Vera B. Dolgopel, M.D., and Helen Y. Markus, M.T., entitled "Rapid Diagnostic Method for Testing the Virulence of Corynebacteria," the second sentence under *Virulence Test* should read:

"A similar amount was injected *intracutaneously* into another guinea pig which was used for the same number of control tests, and 500 units of diphtheria antitoxin was given *intraperitoneally*."

DEPARTMENT OF ABSTRACTS AND REVIEWS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

SYPHILIS, Latent, A Serologic Verification Test in the Diagnosis of, Kahn, R. L. Arch. Dermat. & Syph. 41: 817, 1940.

A serologic test is presented in this article which should help to detect false positive reactions obtained in the serodiagnosis of syphilis. The test is based on experimental studies which indicate that sera of nonsyphilitic persons which give positive reactions possess characteristics that can be differentiated from those possessed by sera of syphilitic persons. The technique of the test is similar to that of the standard Kahn test, except that it is carried out at 37° C. and at 1° C. The technique is applicable in questionable cases of syphilis in which sera give doubtful or positive reactions with a diagnostic test.

The method follows:

In the performance of the verification test, a thorough knowledge of the Kahn test is necessary. In a broad sense, the verification technique consists in performing Kahn tests at 37°, 21°, and 1° C. Since such tests are routinely carried out at approximately 21° C., the basic differential temperatures are 37° and 1° C.

In carrying out the technique at 37° C., the reagents as well as the glassware must be properly warmed, and the reagents must be mixed under warm conditions. 1. Kahn racks and tubes are warmed by placing them in a 37° C. water bath fifteen minutes before performing the test. The pipettes are kept in a 37° C. incubator. 2. Standard Kahn antigen is mixed at room temperature in the usual manner with saline solution according to the titer. The antigen suspension is then transferred to a Kahn tube, stoppered and placed in the water bath for fifteen minutes. 3. The serum is heated for thirty minutes at 56° C. (132.8° F.) and is then placed in the 37° C. water bath for fifteen minutes before being pipetted into the antigen suspension. 4. The amounts of antigen suspension (0.05, 0.025, and 0.0125 c.c.) are deposited at the bottom of the Kahn tubes, followed by the serum (in 0.15 c.c. quantities), and the mixture is shaken for ten seconds without removing the rack from the water bath. The racks remain in the bath from five to ten minutes and are then shaken for three minutes under standard conditions. 5. Physiologic solution of sodium chloride, kept in a 37° C. water bath, is then added to each tube in the usual amounts (1, 0.5, and 0.5 c.c.); the tubes are agitated sufficiently to assure proper mixing, and the results are read immediately. 6. A microscope mirror or a slit lamp is used in the reading of results. The precipitation readings in each tube are recorded in the usual manner.

The technique at 1° C. requires an ice-water bath, which can be easily improvised with chopped ice. A square, flat-bottomed pan, 6 inches (15 cm.) or more deep and large enough to hold several racks, will be found adequate. Every step in the technique as carried out in the 37° C. water bath is applicable to that in the 1° C. water bath. It is important not only to bring the temperature of the serum and antigen suspension to 1° C. but also to see to it that the serum is added to the antigen suspension and mixed at this temperature. Tests performed at 1° C. may require special care in reading owing to the film of moisture that condenses on the outside of the tubes. This film may be wiped off with a cloth and the results read as previously indicated.

In the experiments described in this article the three-minute shaking of the tubes was carried out at room temperature. In experiments to be undertaken it is planned to determine the effects of shaking at 37° C. and at 1° C.

It is suggested that no more than ten tests be carried out at any one time, either at warm or at cold temperatures.

In view of the fact that the basic value of the test under consideration lies in the difference in the precipitation results at 37° and 1° C., it is evident that technical steps which do not adhere strictly to these two temperatures will neutralize the value of the results.

GLUCOSE, Renal Threshold for; Normal and in Diabetes, Lawrence, R. D. Brit. M. J. May 11, 1940.

The study of the renal threshold in diabetic and normal persons with healthy renal function shows frequent upward and downward deviations from the accepted "normal" figure of some 170 mg. per 100 c.c.

The conception of a "normal" threshold is false. There is an average renal threshold, just as there is an average blood pressure, and the many deviations from it should be looked upon as physiologic—a matter of individual idiosyncrasy, and of no pathologic significance.

High and low thresholds are frequent and usually persist at the same level throughout life. A few factors outlined above affect the threshold temporarily or even permanently.

The stigma of something abnormal (and hence pathologic) that has been imputed to a high or low renal threshold should be removed.

BRUCELLA: The Presence of a Capsule on Brucella Cells, Huddleson, I. F. J. Am. Vet. M. A. 96: 708, 1940.

Capsules have been demonstrated by the method following:

A dilute suspension of Brucella cells is prepared in physiologic salt solution or distilled water. A small drop of the suspension is placed near one end of a clean microscope slide, and drawn across the slide by placing the end edge of another slide at one angle in front of the drop. The film of bacteria is dried in the air. A small drop of Keuffel and Esser India drawing ink No. 3000 is placed back of the beginning of the previous smear and drawn over it with the end edge of another slide. The ink smear is allowed to dry in the air. The smear is now treated with methyl alcohol containing 0.2 per cent glacial acetic acid for five seconds and again air-dried. The slide, film up, is placed on a rack and stained five minutes with aniline crystal violet previously diluted 1:1 with distilled water. The excess stain is now washed off with distilled water and the smear is air-dried.

On examining the smear under the microscope (oil-immersion objective, 10 or 12 × ocular), it is necessary to move the slide to an area where the ink smear is not too dense in order to view the encapsulated bacteria in their normal size. The soma appears as a deep purple coccus or rod inside the capsular material, which stains light pink or purple. If the smear is fixed for five seconds with 5 per cent acetic acid prior to staining, the capsular material does not stain but appears as a light halo around the soma or bacterial body. The size of the capsule depends upon the size of the soma. The size varies from 1.5 to 2.5 microns.

The capsule appears larger in recently isolated cultures and in those that have grown on culture media for not longer than seventy-two hours.

Cultures that have become completely dissociated or rough do not show a capsule.

WEIL'S DISEASE in Hawaii, Tokuyama, S. J. A. M. A. 114: 2195, 1940.

Twelve cases of hemorrhagic spirochetel jaundice were observed in Hawaii, the diagnosis being confirmed clinically in two cases and bacteriologically in nine cases. The disease was proved to be identical with that occurring in Japan by both clinical and bacteriologic criteria.

Nine of the twelve patients were inoculated with the *Leptospira icterohemorrhagiae* serum of Inada and Ido; seven of the inoculated patients were cured and two died. Death was due to the delay of the serum injection.

Three of the patients did not receive the serum; of these, two died but one recovered.

DARKFIELD, A New Technic for, Sizemore, T. *Am. J. M. Technol.* 6: 120, 1940.

The ulcer or lesion to be examined is cleansed carefully with sterile normal saline or Ringer's solution, removing all debris and pus. The ulcer is then dried by the application of ether. A sufficient amount of serum for examination may be obtained by exerting general pressure upon the sides of the ulcer or lesion. Place the top of an ordinary blood coagulation pipette to the serum and tilt until enough of the serum is drawn into the pipette. Transfer to a clean slide using a small rubber bulb to expel the serum. Place a cover glass over the serum immediately and compress it slightly. Mount as usual in cedarwood oil.

URINE, The Formation of, Edwards, J. G. *Arch. Int. Med.* 65: 800, 1940.

In the kidneys of all classes of vertebrates the proximal convolution is primarily secretory and is the only part of the tubule invariably present. The structure and functions of the tubule, when viewed phylogenetically and when studied in health and in disease, would seem to have an importance much greater than is at present demonstrable in detail. Comparative renal studies dealing with the aglomerular kidney, and inaugurated by the author in this connection, have been productive of much interest in secretion by the tubule. It is hoped that eventually the actual role of the tubule in urine formation will be established.

The phylogenic development of the corpuscle as the site of filtration is related to that of the distal convolution as the site of resorption. Filtrable substances in the plasma are excreted by the corpuscle regardless of possible depletion of body fluids and consequent damage to the tissues. The distal convolution, aided in birds and mammals by the thin segment and the ascending limb of the medullary loop, performs by resorption the function of salvage. The hypotonic urine of cold-blooded animals indicates that there has been resorption of relatively more of certain solutes than of water; the hypertonic urine of warm-blooded animals indicates the opposite.

A filtrable fraction of plasma is excreted into the lumen of the tubule by the corpuscle and by the cells of the proximal convolution. Minimal amounts of protein are present in this fraction because the passage of larger amounts is prevented by the epithelial wall adjacent to the glomerular and peritubular capillaries. Many factors so affect filtration by the corpuscle, however, that albuminuria readily develops. The extreme sensitiveness of this structure to such normal or pathologic changes as those which occur in the chemical composition of plasma or in blood pressure flow or volume is responsible for confusing variations and dubious interpretations of its activity.

Diuretics are initially and chiefly effective by their direct or indirect action on one or more of the components of the cardiovascular system. Neither diuretic urine nor the subnormally concentrated urines formed after renal injury should be regarded as necessarily indicating impairment of the resorptive power of the tubule. Urines of low specific gravity may be variously formed as a result of excessive filtration, of decreased resorption of water, or of increased resorption of solutes.

It is not yet established that the formation of urine is primarily the result of filtration and resorption or that resorption is the chief function of the tubule as a whole.

SHOCK: Circulatory Failure of Capillary Origin, Moon, V. H. *J. A. M. A.* 114: 1312, 1940.

Circulatory failure of capillary origin produces a clinical syndrome which is highly characteristic. This is accompanied by an equally characteristic group of abnormal physiologic and biochemical features. These constitute the clinical syndrome of shock.

The gross and microscopic changes seen post mortem are equally significant. These consist of evidences of capillary damage, such as dilatation, stasis, petechial hemorrhages, edema, and effusions, present in extensive visceral areas. These features are etiologically related to the mechanism by which circulatory deficiency of capillary origin develops.

Hemoconcentration is a highly valuable clinical sign indicating the early stages of this condition. It occurs before other signs of circulatory deficiency are manifest.

The mechanism of this type of circulatory failure includes the reciprocal effects of two major factors—capillary atony and tissue anoxia. Either of these factors will presently cause the development of the other. This reciprocal action gives the circulatory deficiency a self-perpetuating quality which tends toward an irreversible condition.

Few conditions of disease present a more characteristic group of clinical features, physiologic abnormalities, and morphologic visceral changes. This syndrome, if caused by a single etiologic agent, would constitute an entity among diseases. But the origin of this syndrome is highly diversified. It may originate from the numerous and varied agents and conditions which may injure directly the capillary endothelium, from loss of blood and/or fluid sufficient to produce systemic anoxia, from agents or conditions which reduce the volume flow of blood below physiologic limits, from asphyxia of external or internal origin, or from various combinations of the conditions mentioned.

Efforts to combat this type of circulatory deficiency will be directed toward the recognition and removal of the cause and toward interrupting the operation of the vicious circle. If the former cannot be accomplished, the latter will be ineffective.

Until some agent is found which will prevent or relieve capillary atony, efforts to interrupt the cycle should be directed toward the restoration of blood volume and toward relieving the anoxia. The tendency to progress toward irreversibility requires early recognition and action. Hemoconcentration is recommended for recognizing the early stages of circulatory deficiency of this type.

URINE: Control of Urinary Reaction, Bridges, M. A., and Mattice, M. R. *Am. J. M. Sc.* 200: 84, 1940.

The urinary pH cannot be consistently changed at will without regard for its natural trend.

The acidifying or alkalinizing effect of test substances cannot be judged solely by the urinary pH observed. Comparison with the fundamental or natural curve is necessary.

The natural acid-base curve can be distorted by dietary factors with variable ease in different subjects.

The determination of urinary pH following meals is of no practical clinical value.

It has not been possible by dietary measures to produce continuously alkaline urine.

Temporary elevation of urinary pH is most readily secured with cantaloupe.

The easiest means of maintaining acid urine in the normal individual is the substitution of cranberry juice for water as such in the diet.

The effective action of acidifying drugs is conditioned by the amount of water simultaneously claiming excretion.

Although concentration of urine is regularly associated with increasing acidity and dilution with increasing alkalinity, the normal kidney can excrete a highly acid dilute urine and a definitely alkaline concentrated urine. The elimination of water is not constant throughout the day, thus influencing the urinary pH.

Emotional and mental states demonstrably affect urinary pH.

PNEUMONIA, Sabin Agglutination Test as a Control of the Sulfapyridine Treatment of
Fox, W. W., Rosi, R., and Winters, W. L. *Am. J. M. Sc.* 200: 78, 1940.

Fifty adult patients with pneumococcal lobar pneumonia treated with sulfapyridine were studied to determine the time at which active immunity first appeared.

All patients with proved type-specific pneumococcal pneumonia developed strongly positive agglutinins at some time in the course of their illness.

Strong type-specific agglutination occurred in the uncomplicated cases on an average of 8.3 days after the onset of the disease; in complicated cases it occurred on an average of 12.5 days.

When active immunity did not appear early in the second week of the disease, complications or delay in resolution frequently occurred.

Persistence of a strongly positive agglutination for the homologous type pneumococcus was a favorable prognostic sign.

Occasionally, empyema developed after a strongly positive agglutination appeared. All such cases, however, had bacteriemia on admission and in some cases this persisted for twenty-four hours or longer after sulfapyridine therapy was begun.

The authors believe that recovery from pneumonia in patients treated with sulfapyridine requires: (1) adequate blood sulfapyridine concentration, (2) maintained leucocytosis, and (3) development of active immunity by the patient in response to his infection.

In uncomplicated pneumococcal pneumonias a safe rule appears to be to continue sulfapyridine therapy at a dosage which will produce a blood level above 4 mg. per 100 c.c. until a strongly positive microscopic agglutination test is obtained with the patient's serum against pneumococci of the type with which he is infected. In complicated pneumococcal pneumonias sulfapyridine should be continued, if possible, until the temperature and pulse have returned to normal, even though strongly positive agglutination has occurred prior to that time.

TRICHINOSIS, Incidence of, in 300 Autopsies at the University Hospital, Ann Arbor, Michigan, Catron, Lloyd. *Am. J. Hyg.* 1: Sec. C, 1940.

Forty-four instances of trichinosis (14.7 per cent), all unrecognized clinically, were found by examination of diaphragms obtained from 300 autopsies at the University of Michigan Hospital. All the infestations were shown by digestion of samples of 50 Gm. or less; three were found by examination of a single microscopic section from each diaphragm; and eight were found in the 270 cases in which 1 Gm. of diaphragm was examined microscopically. The digestion method, although demonstrating every infestation, proved inadequate to show the total number present when the larvae were dead and calcified.

TISSUE: Pathologic Changes Observed in Human Tissues Subjected to Subcritical Temperatures, Smith, L. W. *Arch. Path.* 30: 424, 1940.

In this paper a brief summary of the pathologic studies in a series of some 60 cases in which patients with cancer were subjected to local or general reductions of temperature and in which autopsies were made is presented. The studies are divided into three major groups: first, those relating to the normal body tissues; second, those relating to tumor tissue subjected locally to temperatures of 40° to 50° F. for varying periods of time; and, third, those relating to metastatic tumor tissue in patients whose general temperature had been reduced to subcritical levels between 74° and 90° F.

Relating to the first group of non-neoplastic or "normal" tissues, it may be said "a priori" that significant changes are the exception rather than the rule. In the series of 60 autopsies marked myocardial degeneration was found in 5 patients. In 3 of these it was associated with definite sclerosis of coronary arteries. In 3 patients acute pancreatitis was seen; these patients had been accustomed to take relatively large amounts of morphine. In 4 patients a significant and persistent fall in blood pressure was observed. In 3 of these there was extensive bilateral adrenal metastasis. Bronchopneumonia other than the terminal event commonly encountered in cases of advanced malignant disease appeared chiefly in the postrefrigeration period, in association with metastatic involvement of the lungs. Its occurrence with and its relationship to refrigeration, accordingly, is particularly difficult to evaluate.

With respect to the second group, temperatures of 40° to 50° F. applied locally to tumors regularly produced regressive changes going on to actual necrosis, even to the point of histologic clearance of the tissue of tumor cells in occasional instances.

In regard to the third group, the changes which were observed in metastatic tumor tissue from patients who were submitted to general reductions of temperature to 74° to 90° F. were similar in kind but varied greatly in degree in comparison with those resulting from application of the lower temperatures locally. In no case were regressive changes encountered until ninety-six to one hundred and twenty hours of refrigeration had been given, and in some cases no significant change seemed to occur even after three hundred hours of such low temperatures.

HYDATIDIFORM MOLE, Genesis of, Hertig, A. T., and Edmonds, H. W. Arch. Path. 30; 260, 1940.

A series of 1,027 spontaneously aborted ova and 74 hydatidiform moles has been studied with respect to the genesis of hydatidiform degeneration. Hydatidiform degeneration to some degree is common, occurring in 40 per cent of these spontaneously aborted ova, or in 4 per cent of all pregnancies. Typical stages in the evolution of a hydatidiform mole are described. A classic hydatidiform mole is uncommon, occurring only once in 2,062 full-term deliveries in this clinic.

Pathologic ova, of ten and two-tenths weeks' mean menstrual age, constituted 47.4 per cent of the total number of spontaneously aborted ova; 66.9 per cent of the pathologic ova showed early hydatidiform degeneration of the chorionic villi. "Nonpathologic" ova, of fifteen and four-tenths weeks' mean menstrual age, constituted 52.6 per cent of the total; 11.6 per cent of such ova showed early hydatidiform degeneration of their villi.

Hydatidiform degeneration of the chorionic villi of pathologic ova begins, in all probability, at about the fifth week of pregnancy, the time when the fetal circulation should begin to function. The fetal circulation in the chorion of the pathologic ovum fails to function because of extreme defectiveness or absence of the embryo. The vascular "anlagen" disappear coincidentally with the onset of hydatidiform degeneration, both processes being a function of absence or of defectiveness of the circulation.

Hydatidiform degeneration is prone to occur in the villi of early pathologic ova because the stroma of the villi is normally loose and their chorionic epithelium is normally active. Hydatidiform degeneration in the villi of early pathologic ova is an expression of continued physiologic activity of the trophoblast (absorption and/or secretion), with resultant accumulation of intravillous fluid, which cannot be utilized because of the lack of a functioning fetal circulation.

Hydatidiform degeneration is less likely to occur in "nonpathologic" ova because of a functioning fetal circulation of some weeks' duration, relatively dense stroma, and relatively inactive chorionic epithelium.

Transitional moles, of sixteen and six-tenths weeks' mean menstrual age, are usually typical pathologic ova containing no embryos, or at most extremely defective ones, although a few contain macerated normal embryos. Typical hydatidiform moles, of seventeen and four-tenths weeks' mean menstrual age, are true pathologic ova possessing no embryos or rarely a very defective one. A typical hydatidiform mole is, therefore, derived from a true pathologic ovum in which the embryo was either absent or very defective from the beginning and which, for reasons unknown, failed to abort at the usual time. Hence, it constitutes a type of "missed abortion."

BLOOD, Significance of Polymorphonuclear Toxic Index in Affections of the Musculo-Skeletal System, Sutro, C. J. Bull. Hosp. for Joint Dis. 1: 61, 1940.

In a survey of approximately 8,000 patients with affections of the musculo-skeletal system, it was found that, in certain cases, the estimation of the toxic index gave additional clinical information, especially in regard to differential diagnosis and prognosis. Since the evaluation of the toxic index is a simple procedure, its application to problems of differential diagnosis and prognosis is warranted.

(The index is equal to number of polymorphonuclear cells showing toxic granules divided by total number of polymorphonuclear cells and the product multiplied by 4.)

REVIEWS

Books and Monographs for Review should be sent direct to the Editor,
Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

Experimental Poliomyelitis*

THE neutralization test is a method for determining the ability of immune serum to inactivate virus in vitro—in this instance, that of poliomyelitis. In their thorough review of the literature, the authors point out that though much information can be obtained from the test, the discrepancies and irregularities in results interfere with its effective application. The test is of practical value where qualitative determinations are desired, but it is unsuitable for quantitative studies.

In the second portion of the book, which is given over to a description of the author's carefully controlled experiments, a search was made for sufficient data for the development of a more refined and standardized technique. Unfortunately, their goal was not attained.

The final conclusions of the authors are that the test is a specific antigen-antibody reaction, but its presence does not necessarily imply resistance to infection. Since with the test in its present form quantitation is not a practical procedure, improvement or replacement of the neutralization test is desirable for the advancement of immunologic knowledge in poliomyelitis.

Neoplastic Diseases†

THE fact that this classic has now come into its fourth edition attests to the position it holds in the field of medical texts. The author has considerably revised many sections and completely rewritten some chapters.

The opening section on general oncology should be read by all doctors who have occasion to see malignancies, whether they be primarily interested in medicine or in surgery. The chapters on the nature of cancer and on experimental cancer research are particularly interesting.

The second, larger section on special oncology is a most complete and scholarly treatise on tumors of all types.

A book that should be in every physician's personal library, it is of equal value to the medical student, the internist, the surgeon, and the pathologist.

An Introduction to Biochemistry‡

IT IS of distinct benefit to the clinician from time to time to read through a book of this caliber. Although a small volume, it is comparatively comprehensive. The point of view entertained throughout is that biochemistry is an independent discipline related to the physiologic group on the one hand, and pure chemistry on the other.

*Experimental Poliomyelitis. I. A critical review of the literature with special reference to the use of the neutralization test in immunological studies. II. The Neutralization Test. A study of some factors involved in the neutralizing action of immune serum against poliomyelitis virus. By Morris Schaeffer, Ph.D., and Ralph S. Muckenfuss, M.D., Department of Health, Bureau of Laboratories, New York, N. Y. Cloth, 158 pages. Published under the Auspices of The National Foundation for Infantile Paralysis, 1940.

†Neoplastic Diseases. A Treatise on Tumors. By James Ewing, A.M., M.D., Sc.D., LL.D., Professor of Oncology at Cornell University Medical College, New York City; Consulting Pathologist, Memorial Hospital. Cloth, ed. 4, 1,160 pages, revised and enlarged, with 541 illustrations. W. B. Saunders Co., Philadelphia and London, 1940.

‡An Introduction to Biochemistry. By William Robert Fearon, M.A., Sc.D., M.B., F.I.C., Fellow of Trinity College, Dublin, Member of the Royal Irish Academy. Cloth, ed. 2, 415 pages, The C. V. Mosby Co., St. Louis, Mo., 1940.

After a rapid review of pertinent aspects of inorganic chemistry, the author turns to a more detailed study of organic compounds. Analytical methods are afforded their place in the discussion of each substance.

Written in an easy style, the book is graced by classical quotations some of which are especially apt. Over the index for instance, one sees "how index-learning turns no student pale, yet holds the eel of science by the tail" (Pope). This book may be heartily recommended.

Edible Wild Plants*

IF I AM ever lost in the forest and must subsist on what foods may be had from the wild plants about me, I shall want to have this book with me. It will tell me how to recognize and identify those wild plants which are edible, thus avoiding those which may be highly poisonous.

I will find a large variety for my menu, which will include 69 species of edible fruits and berries, 26 of salad plants and potherbs, 19 of roots and tubers, 15 of nuts, 13 of beverages and flavoring plants, 10 of seeds and seed pods, all minutely described, and a large number of others more briefly listed. Not only that, but if I am so fortunate as to have the book with me, I will find directions how to prepare or cook these plants.

There was a time when our pioneering ancestors realized the vital importance of this information. Now, when we are all eating cultivated foods, the knowledge has been all but lost.

Dr. Medsger has spent thirty years assembling the information recorded in his volume. It is profusely illustrated. It should be equally valuable to botanists and to those whose primary interest is in foods.

Compendium of Regional Diagnosis in Lesions of the Brain and Spinal Cord†

ACH year sees new books in medicine, each representing a new approach or interpretation. When the author's novel concept is good and is well grounded, the volume achieves popularity. With the lapse of time, others working in the same field may have better, more modern or more practical ideas concerning the presentation of the subject under discussion. When this occurs, the earlier texts, no matter how excellent they may have been, may pass into eclipse.

Some of them so adequately fulfill the need for which they were prepared that they outlive volumes which are submitted as more modern and may have been designed to supplant them. Such a work is Bing and Haymaker's regional diagnosis. After thirty years it still remains the preferred *vade mecum* for students for neuro-anatomy and neurologic localization.

There are several reasons. The authors' original manner of presentation filled a need. This having been achieved, there has been no attempt to pad successive editions, or to cover additional and less pertinent aspects. The book is still a small volume. The illustrations, many of which are in color, are quite completely descriptive and are sufficiently numerous to cover all phases of the subject. The general make-up of the book facilitates easy reading.

This volume is equally valuable to the freshman student learning neuro-anatomy, the neurologist and neurosurgeon, and the internist and general practitioner who require a ready reference work dealing primarily with the localization of lesions of the central nervous system.

*Edible Wild Plants. By Oliver Perry Medsger, Professor Emeritus of Nature Education, Pennsylvania State College. With an Introduction by Ernest Thompson Seton. Cloth, 225 pages, illustrated with 80 pen and ink drawings and 19 photographs, \$3.50. The Macmillan Co., New York, N. Y., 1939.

†Compendium of Regional Diagnosis in Lesions of the Brain and Spinal Cord. A Concise Introduction to the Principles of Localization of Lesions of the Nervous System. By Robert Bing, Professor of Neurology, and Webb Haymaker, Assistant Professor of Anatomy, University of California. Cloth, ed. 11, 292 pages, with 125 illustrations, 27 in color, and 7 plates, The C. V. Mosby Co., St. Louis, Mo., 1940.

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PROGRESS

FAMILIAL NONREAGINIC FOOD ALLERGY AS A PREDISPOSING CAUSE OF COMMON COLD

ARTHUR F. COCA, M.D., ORADELL, N. J.

THAT nonspecific influences may act as predisposing causes of common cold has been amply demonstrated in two of a brilliantly conceived series of studies on "Nonspecific Factors in Resistance" that have been carried out by Arthur Locke. These remarkable papers have had such scant attention in medical literature, and the conclusions reached in them are so directly applicable to the observations about to be described, that a summary of the outstanding pertinent results of Locke's findings is included here as follows:

SUMMARY OF LOCKE'S INVESTIGATIONS

1. Normal rabbits differ in the speed with which they can accomplish removal from the circulating blood of small numbers of intravenously injected type I pneumococci. Only those rabbits whose rate of removal of the injected pneumococci is faster than the rate of proliferation of the pneumococci survive the injection.
2. When normal rabbits are immersed in cold water until the body temperature has been reduced from the normal 102° to 103° F. to between 95° and 96° F., they differ according to the time required for a temperature recovery of 3° F.—"warming time." Those with fast recovery (warming times of 30 to 33) dispose of injections of small numbers of virulent type I pneumococci with a rapidity that permits a high percentage of survival. On the contrary, rabbits with a slow recovery (warming times longer than 45) are unable to remove the bacteria from the blood at a rate sufficient to assure survival.¹⁻⁴

As Locke points out, these methods of study are not applicable to man, but in his study of common cold he has made use of two other methods with

¹From the Lederle Laboratories, Inc., Pearl River, N. Y.

which he has obtained results that carry the same significance in principle as those of the rabbit experiments just described.

Locke determined the "oxygenation time" in a group of persons variously susceptible to common cold under conditions of physical stress which were produced by having the individual undertake a vigorous exercise of the muscles of the back, arms, and legs in a specially designed apparatus.⁵ The "oxygenation time" represents the rate at which oxygen is used up by the individual when he exerts himself to the utmost in the apparatus; the figure thus obtained is corrected according to the estimated body surface of the person.

In the "optimal response" A the "oxygenation time" was in the neighborhood of 0.60; it was shorter, e.g., 0.40 to 0.44, in the "hyperresponse" C' and longer, e.g., 0.79 and 0.85, in the "hyporesponse" C.

Under this procedure Locke found that among those persons classified as A ("optimal response") 74 per cent reported less than two colds during the year, whereas among the C group only 5 per cent had had less than two colds during the year.

Locke found also that, regardless of the classification determined in the individual previous to the onset of a cold, the grading at the time the attack began was C, and the condition of the person was that of "physical exhaustion and shock which a grading of C connotes." He states: "Common cold has less similarity to infections of the type producing small-pox, typhoid and diphtheria than with infections of the type observed as a result of invasion from the lungs and intestines in dogs and rabbits during transient exhaustion⁶ and shock."⁷

In a more recent study⁸ Locke has confirmed his general thesis that "low-grade infection (such as common cold) gains foot-hold because of impairment in the ability of the defense mechanisms to function." In this study he has used a method which spares the individual the physical strain that was involved in the determination of the oxygenation rate. This method requires the person merely to inhale 5 per cent carbon dioxide for one to two minutes. The inhalation of 5 per cent carbon dioxide causes a "measurable, reproducible and characteristic increase in the depth of breathing."

Defining the "ventilation quotient" as the ventilation rate, noted under the influence of carbon dioxide, divided by the basal ventilation rate (B.V.R.), as observed when the person is merely lying at rest, Locke proposed the figure 1.29 as the optimal ventilation quotient of a normally resistant individual. A group of persons showing ventilation quotients within 5 per cent of this optimum showed a much lower incidence of common cold than did the group showing ventilation quotients diverging 10 per cent or more from that optimum. One may again sum up Locke's results with his two methods as follows: there is a state of impaired physiologic function, the nature and cause of which is unknown, that can be detected in the individual through two quantitative methods; this state of impaired physiologic function may be found to affect some individuals at one time but not at another. Those individuals in

whom the impaired function is not found are less susceptible to colds than are those in whom it is found, and the impairment is always present at the onset of a cold.

These findings indicate that susceptibility to common cold is not due to a lack of the mechanism of defense but to a nonspecific impairment of the ability of the mechanism to function.

For a certain purpose, unrelated to the present inquiry, I made in September, 1939, a survey of 825 employees of Lederle Laboratories in Pearl River, N. Y., with respect to the incidence of common cold. These persons were kept under periodic observation in the winter and spring of 1939-1940. Among the 825 individuals 99 reported having had no colds in the past three years. Locke in Pittsburgh finds 12 per cent of unselected persons free of colds.

Fifty-two of these cold-free persons were selected for further examination because of their ready accessibility and their intelligence. Fifty-one persons who had a history of at least one cold* annually were similarly selected for examination.

Each individual of these two groups was repeatedly questioned as to the presence of food allergy† in himself and in his immediate family. In a few instances, when the inquiry took place shortly after a meal, the pulse rate was observed. This latter evidence was helpful, of course, only when the rate was high enough to be considered probably food allergic. The results of this examination are shown in Tables I and II.

Among the group of 51 persons who were subject to colds, there were three (W. K., W. Kw., and A. W.) who presented no personal history of food allergy; however, the high pulse rate of 96 observed in two of these men one hour after breakfast marks them as most probably food allergic. N. G., J. H., and P. P. gave a bilateral negative family history of symptoms of food allergy, yet there is no doubt that all three are themselves food allergic. The strongest point of evidence of food allergy in C. C. is the bilateral family history of food allergy. The evidence of food allergy in H. H., B. R., and E. W. is weak.

Acknowledging the possibility that A. W., C. C., H. H., B. R., and E. W. were not food allergic, one may draw from these data the conclusion that a large proportion, probably about 90 per cent, of cold-susceptible persons are food allergic. Among the 52 persons who had had no colds in the seasons of greatest risk of 1939-1940, and who reported having had no colds in the previous two years, 31 reported a negative family history, as contrasted with three such persons among the cold-susceptible group. Two others had sisters or brothers all of whom were free from food-allergic symptoms. Five others

*If the question should arise as to whether some of the group of cold-susceptible persons were individuals with perennial allergic coryza, it can be pointed out that since the group was not self-selected, but chosen at random, not more than 2 per cent (at most one or two individuals) could be expected to be so affected. Incidentally, one of the cold-free group has had allergic coryza in the winter, which has been controlled by specific treatment. This employee never mistook her condition for common cold.

†The cold-susceptible persons presented typical histories of an acute infectious coryza both as to clinical course and as to the characteristic change in the exudate.

†The expression "food allergy" throughout this paper refers only to familial nonreaginic sensitivity to food, a category which, according to evidence that will be presented in a later paper in this JOURNAL, must be separated from reaginic atopy (asthma, hay fever, atopic dermatitis).

TABLE I—CONT'D

J. G.	Several	84	x	x ^r	x	x	x	x	x	x	x	x	x	x	x	M headaches Bilateral Negative Parents ?; sister positive Unilateral Bilateral Bilateral M headaches, neuralgia Unilateral M hives Bilateral Unilateral ? Unilateral Negative ? ? ? M ?; F headaches ? M heartburn, tiredness Bilateral F ?; M positive Bilateral M positive Bilateral M positive Bilateral M headaches M asthma; sibs indigestion ?	Sister has headaches, heartburn, dizziness, nervousness
T. H.	Some																
J. H.	1																
P. H.	Some																
J. H.	3 or more																
F. J.	Some	96															
W. K.	1	96															
W. Kw.	1																
R. K.	2 ¹																
H. L.	Some ^a																
N. L.	Some																
E. M.	2																
H. M.	2 or more																
S. M.	Some	99															
P. P.	1	108															
J. P.	Some																
A. R.	Some																
D. S.	Some																
T. S.	Some																
M. S.	Some																
J. T.	Some																
C. T.	Some	100															
E. V. L.	Several																
A. V. V.	Some																
H. Z.	2																
A. W.	2 or 3	72															
C. C.	1	72															
H. H.	1	68															
B. R.	Some																
E. W.	2																

¹M = mother; F = father.²Sore throats.³Oc = occasionally.⁴Leg.⁵Nausea.⁶Crab.⁷Orange.⁸Also boils.⁹Facial.

Patient has dermatographia and constipation

Probably not food-allergic
Questionably food-allergic
Questionably food-allergic
Questionably food-allergic

Questionably food-allergic

TABLE II
INCIDENCE OF FOOD-ALLERGIC SYMPTOMS IN PERSONS NOT SUSCEPTIBLE TO COLDS

NAME	COLDS LAST 3 YEARS	PUSE RATE 1 TO 2 HOURS AFTER MEAL	HIVES	HEADACHES	HEARTBURN	INDIGESTION	CANKER SORES	DIZZINESS	DIARRHEA	NERVOUSNESS	NEURALGIA	PHYSICAL TIREDNESS	FAMILY HISTORY PARENTS OR SIBS	REMARKS
B. P.	None												Unilateral	
F. P.	None												Negative	
A. R.	None												Negative	
S. R.	None												Negative	
H. S.	None												Negative	
W. S.	None												Negative ?	
W. S.	None												Negative	
J. V.	None												Negative	
Mrs. J. V.	None												Negative	
D. V. H.	None												M negative; F ?	
W. W.	None												M negative; F ?	
J. R.	None												Negative	High blood pressure
J. K.	None												Negative	
E. M.	None	56											Negative	3 sisters negative
A. S.	None												Negative	3 sisters negative
M. M.	None												Negative	
A. S.	None			x ³									Negative	Asthma
B. K.	None			x ³									M ?; F negative	Sore throat this winter; no cold
G. S.	None												Negative	Father gassed twice
C. L.	None												Negative	Three years ago bronchitis and pleurisy
M. A.	None												?	3 sibs negative; wife and son (aged 18 years) headaches
F. R.	None												?	Hay fever
J. B.	None												M dizziness, nervousness; F negative	

reported a negative history in one parent. Twelve were unable for one or another reason to inquire of either parent about the matter, and only two gave a bilaterally positive family history of food allergy, as contrasted with eleven such persons in the cold-susceptible group.

The last seven of the cold-free group are no doubt food allergic, but mildly so, excepting possibly A. S. and E. W. The preceding four are questionable with regard to food allergy.

The comparison of the data in Tables I and II hardly permits any doubt as to the important role of food allergy in predisposing the person to common cold. Granting this conclusion, one can now appreciate fully Locke's application to his problem of the scientific method, in both design and technique, and also the accuracy of his deductions.

Throughout his study Locke seems to have been quite in the dark as to the nature of the original causes of the "nonspecific factors" of whose existence he was collecting such convincing evidence. Although the thought of allergy as a possible nonspecific influence evidently crossed his mind, it seems to have been set aside, no doubt because he was not aware of the large incidence of that state and had no method of detecting its presence.

Particularly illustrative of his correct insight is his forthright assertion "Probable nonspecific predisposition to common cold, at the time of test, is not identical with the probable future incidence." In the light of the result of our own investigation, this statement may be paraphrased as follows: the food-allergic state, which usually predisposes to common cold, may be present at the time of test in some persons who are not cold susceptible (see last cases in Table II).

For the question: What is the nature of the nonspecific predisposing cause of common cold? there is a suggestion that arises out of a very commonly observed phenomenon in food-allergic persons: namely, the rapid increase and equally rapid loss of weight which sometimes is noticed at the onset of a allergic headache and as the attack passes off. Some observers have reported that the loss of weight has been associated with an increase in the excretion of urine.

I have noticed this phenomenon in several persons with food allergy who symptoms were relieved by avoidance of the allergenic foods. In two persons, J. and A. P., urticaria was an outstanding symptom. After the allergenic foods were identified and eliminated, there was a permanent loss of several pounds in weight, although the diet was ample and well balanced and the general health was decidedly improved in both individuals. The rapid loss of weight in these cases is, of course, due to the withdrawal of fluid from the edematous tissues similar to that described by von Pirquet and Schick in the recovery from serum disease. This phenomenon is not limited to cases of urticaria so-called angioneurotic edema. The allergic infiltration of fluid into tissue is usually so distributed as not to be grossly perceptible.

From the foregoing considerations it is seen that in those cold-susceptible persons in whom food allergy is a predisposing cause of the susceptibility.

"nonspecific factor" of Locke may be the effect of allergic edema. This suggestion has some support in earlier published observations, as interpreted by Hoelzel⁹ and others. A number of investigators¹⁰⁻¹⁵ have reported that the incidence of colds is reduced by restriction of carbohydrate consumption. Hoelzel,¹⁰ McQuarrie,¹² and Higgins¹³ attributed the reduction in the number of colds to an observed lessening of tissue hydration.

There must be other predisposing causes of susceptibility besides food allergy. This conclusion seems forced by the fact that, although between 20 and 25 per cent of white families are free of food allergy,* only 12 per cent are quite free of common cold, and a few of these are food allergic.

SUMMARY

Evidence is offered of the importance of food allergy as a predisposing cause of common cold.

REFERENCES

1. Locke, A.: Lack of Fitness as the Predisposing Factor in Infections of the Type Encountered in Pneumonia and in Common Cold, *J. Infect. Dis.* 60: 106, 1937.
2. Locke, A.: Nonspecific Factors in Resistance. I. Capacity to Sustain Effective Circulation, *J. Immunol.* 36: 159, 1939.
3. Locke, A., and Main, E. R.: Nonspecific Factors in Resistance. II. Ability to Withstand Shock, *J. Immunol.* 36: 173, 1939.
4. Locke, A., Main, E. R., and Mellon, R. R.: Nonspecific Factors in Resistance. III. Capacity for Retarding Bacterial Proliferation, *J. Immunol.* 36: 183, 1939.
5. Locke, A.: Nonspecific Factors in Resistance. IV. The Problem of Common Cold, *J. Immunol.* 36: 365, 1939.
6. Bailey, G. Howard: Accessory Etiologic Factors of Respiratory Infection in Rabbits. II. The Effect of Fatigue on the Susceptibility of Rabbits to Intranasal Infection With Type I Pneumococcus, *Am. J. Hyg.* 9: 192, 1929; Boycott, A. E., and Price-Jones, C.: Experiments on the Influence of Fatigue on Infection, *J. Path. & Bact.* 29: 87, 1926.
7. Boone, T. H., Chase, E. M., and Brink, H. E.: Intestinal Absorption of *B. prodigiosus*, *Proc. Soc. Exper. Biol. & Med.* 29: 113, 1931.
8. Locke, A.: Nonspecific Factors in Resistance. V. Carbon Dioxide Tolerance, *J. Immunol.* 39: 441, 1940.
9. Hoelzel, F.: Diet and Resistance to Colds, *Science* 86: 399, 1937.
10. Hoelzel, F.: Nutritional Edema and Its Relation to the Incidence of Common Colds, *Proc. Soc. Exper. Biol. & Med.* 25: 454, 1928.
11. Hoelzel, F.: A Relation Between the Incidence of Common Colds and Nutritional Hydration, *Science* 68: 301, 1928.
12. McQuarrie, I.: Protein Metabolism of Children on Diets Extremely Low in Carbohydrates, *J. Nutrition* 2: 31, 1929.
13. Higgins, H. L.: Some Physiological and Clinical Effects of High Fat Feeding, *New England J. Med.* 203: 145, 1930.
14. Orr, J. B., and Gilks, J. L.: Studies on Nutrition: The Physique and Health of Two African Tribes, Medical Research Council, Special Report No. 155, 1931.
15. Paton, J. H. P.: Relation of Excessive Carbohydrate Ingestion to Catarrhs and Other Diseases, *Brit. M. J.* 1: 738, 1933.
16. Pickrell, K. L.: Effect of Alcoholic Intoxication and Ether Anesthesia on Resistance to Pneumococcal Infection, *Proc. Soc. Exper. Biol. & Med.* 38: 265, 1938.

*This figure is taken from a survey conducted under the sponsorship of the Board of Health of Oradell, N. J.

CLINICAL AND EXPERIMENTAL

THE DETERMINATION OF BLOOD VELOCITY BY LOBELINE*

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DURING an investigation of the pharmacologic properties of lobeline, the "circulation time" of 95 individuals was determined by this substance. In this communication discussion will be limited to directing attention to a new method of ascertaining the end point of the test and to reporting an additional series of patients studied by this means.

The original contribution of Teplov and Sor,¹ based upon the study of 165 patients, indicated that lobeline provided an objective method for the determination of circulation time. The end point (cough) was striking and brief, the procedure simple, the drug free from toxicity, and the result susceptible to graphic registration. When less than 0.03 to 0.04 mg. of lobeline (or less than 0.08 to 0.12 mg. of synthetic lobeline) per kilogram was employed intravenously, respiration was altered at times; with this dose they noted brief apnea, a cough, and then transient polypnea. Cough was elicited in 60 to 70 per cent of the patients; the percentage of successful results was increased by repetition of the test after 15 to 20 minutes with a slightly larger dose. If cough still failed to occur, the polypnea was registered by a pneumograph in order to obtain an objective end point.

Volgin and Stanojevič² alone observed 49 patients, and with Djordjevič³ they studied 80 individuals. Stanojevič⁴ participated in 500 determinations on 150 patients. Lobeline was used exclusively, and only 7 patients failed to respond with cough; the dosage was individualized but did not exceed 0.07 mg. per kilogram. The circulation time amounted to 10.3 seconds in the average normal adult (Teplov and Sor, 13.4 seconds), to 12.4 seconds in miscellaneous patients; in patients with cardiac compensation it was 13.4 and in those with cardiac decompensation it was 30.2 seconds (Teplov and Sor, 40 seconds). On the basis of 140 determinations Evzlin⁵ concluded that the method was objective, free from unpleasant concomitant manifestations, uniform in its results, and harmless to patients. Trimarchi,⁶ employing lobeline up to 0.18 mg. per kilogram of body weight, reached a similar conclusion. Large amounts seemed necessary in some cases of auricular fibrillation; when the maximal dose was employed, precordial pain and dyspnea developed in some cases, lasted for a few seconds, and vanished without sequelae.

During preliminary experiments arranged to familiarize ourselves with the method, we noted that patients often commented upon a peculiar sensation in the throat ("tickling"); this may or may not be accompanied by cough.

Twenty males whose ages varied from 13 to 64 years and whose weights ranged from 90 to 175 pounds comprised this group. Most of them were suffering from gonococcal urethritis and a few from pes planus. None of them had cough as a symptom. The tests

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were conducted under basal conditions and at the same time of day. Patients imagined they were receiving some therapy. Each of them, regardless of age or weight, received 3 mg. of lobeline intravenously. After the injection they were asked whether they felt anything. If nothing occurred, the test was repeated with 6 mg. of lobeline.

When 3 mg. of lobeline were administered, 9 patients had tickling in the throat, 6 had cough, and 5 had no response. When 6 mg. of lobeline were given to the 9 people who had tickling, 6 responded with cough and 3 with more pronounced tickling. Among the 5 with no response, 3 developed tickling and 2 cough with the larger dose. In short, 15 of 20 patients had an objective response, and the remainder had a reaction which made the end point easily recognized. No ill effects followed.

These results suggest that the intravenous injection of lobeline in doses of from 3 to 6 mg. provides a dependable reaction for the objective determination of circulation in 75 per cent of patients. Employment of an additional subjective symptom practically eliminates negative responses when the larger dose is given.

The circulation time of 75 individuals suffering from various illnesses was then determined.

Alpha-lobeline was employed exclusively. Since 1 c.c. ampoules contain 0.01 Gm., 0.1 c.c. corresponds to 1 mg., and no difficulty is encountered in calculation of dose. Moreover, the volume of the solution is small and the time required for injection is short. Although 5 mg. will usually elicit a response, 3 to 7 mg. were employed in accordance with the weight. (Trimarchi has injected 11 mg. in persons with severe cardiac disease.)

Patients were asked to lie as flat as possible, to relax, and not to hold the breath. The stomach must be empty. The purpose of the test is fully explained to dispel any fear. A tourniquet may be applied to the arm to assist in visualization of the vein, but it should be removed prior to the injection. The injection time should not exceed two seconds. The starting point of the time was placed at the moment the plunger struck the bottom of the syringe; the end point was considered the elevation of the hand or the cough. An ordinary stop watch was employed. A 23-gauge needle, 1 inch in length, was found to be most suitable. No patient experienced any pain; a few times some of the solution spilled into the paravenous tissues but no inconvenience or symptoms resulted. No patient objected to a repetition of the test. Three patients violated the rule of an empty stomach and ate fruit one-half hour before the injection. One did not respond at all; the second responded after fifty seconds, although an eight-second circulation time was obtained on the following day; the third, who had a decompensated cardiac involvement with a prolonged circulation time, showed excessive prolongation (more than one minute), then coughed and vomited.

With small doses (3 to 4 mg.) the rate and depth of respiration may change. However, usually this cannot be employed as an end point since the first alteration of respiration may not be recognizable. Tracings of the respiratory excursion can be secured, but a necessity for any special device defeats general employment of the test.

A peculiar sensation in the throat precedes or accompanies the cough. The occurrence of this sensation may be accompanied by a grimace. The sensation is described in various manners, but usually as tickling or "something coming up quick in the throat." At all events it is perceived as something unusual. Patients were instructed to raise their hand as soon as they felt anything; usually cough occurred while the hand was being elevated. If there is an interval between the two phenomena, not more than one or two seconds separate them.

The cough resembles the explosive cough caused by the inhalation of smoke or dust. It is not severe nor does it distress patients in any way. Individuals suffering from bronchiectasis, pneumoconiosis, or pulmonary tuberculosis, said that the cough was un-

usual and sounded different. Occasionally the patient asked for a sip of water "to fix the throat" which "felt dry." Three patients noted moderate dyspnea; one patient reported a sensation of choking, although no dyspnea was observed. Many individuals experienced an improvement of respiration, were pleased with the injection, and the momentary cough was attributed to nervousness.

No members of the other groups to be reported were normal, but many of them sought treatment for trivial conditions, such as pes cavus and bursitis, which may be regarded as having no bearing on circulation. Physical examination of this group yielded normal findings in the lungs and cardiovascular system. Patients with important affections not involving the heart or respiratory system were also examined. They were considered normal from the cardiovascular standpoint.

In many the venous pressure was estimated by the degree of engorgement of the veins of the neck and the level at which the arm veins collapsed upon elevation of the limb. Exact measurement was regarded as unnecessary. Vital capacity was determined by the usual apparatus, but it did not constitute a part of the test.

Group 1.—Six patients, 5 females and one male, formed this group. Their ages ranged from 22 to 52 years. Four had secondary anemia and tuberculosis, one had a secondary anemia and subacute bacterial endocarditis without cardiac decompensation, and one had pernicious anemia. The hemoglobin estimations in the group ranged from 60 to 65 per cent; the red blood cells did not vary more than 200,000 from three million.

The circulation time in this group of patients who had anemia varied from three and one-half to eight seconds. The two shortest circulation times in the entire series occurred in this group.

Group 2.—Twenty-three patients, 13 males and 10 females, were placed in this group. Their ages varied from 13 to 60 years. Only one patient had a cardiovascular anomaly (cirrhosis of the liver, pulmonary emphysema, and possible beriberi heart. There was some peripheral edema. His circulation time was eight seconds).

The average circulation time in this group of patients who had no anemia, no cardiovascular disease (one exception), but definite illnesses, was 8.3 seconds. One individual (tonsillitis) had a circulation time of twenty seconds but no demonstrable cardiovascular involvement. Individuals with manifest thyrotoxicosis (two observations) had times of five and nine seconds. With the one exception noted the extremes of this group were five to twelve seconds.

Group 3a.—Two males and one female, whose ages varied from 22 to 62, suffered from mild, moderate, and severe pulmonary fibrosis, associated with pneumoconiosis; two of them had electrocardiographic alterations as well. The circulation times of these patients ranged from six to nine seconds. The patient with the lowest vital capacity had the shortest circulation time.

Group 3b.—Three male patients, whose ages were 32, 39, 41, had pneumothorax (one pyopneumothorax). The circulation times ranged from seven to fourteen seconds. The individual with the longest circulation time had a Peabody factor only slightly inferior to that of the patient with a circulation time of seven seconds. There seemed to be no relationship between the Peabody factor and circulation.

Group 3c.—Eleven patients, 8 males and 3 females, comprised this group. Their ages varied from 23 to 79. They suffered from various chest diseases, such as pneumonia, pulmonary

malignancy, and caseous pneumonic tuberculosis. The circulation times ranged from six to ten seconds. The patient with the longest circulation time had pneumonia. The patients with the lowest Peabody factors (3.6, 3.3) had shorter circulation times than a patient with a much higher Peabody factor (5.6). Although cough and dyspnea were present in pulmonary malignancy, the circulation time was eight seconds.

Among 17 patients presenting various diseases of the lungs, the circulation time varied from six to fourteen seconds. Dyspnea of pulmonary origin did not prolong the circulation time.

Group 4a.—Three males and 2 females were placed in this division. Four suffered from rheumatic heart disease, and one from syphilitic aortic valve insufficiency. One patient had cardiac compensation (ten-second circulation time). Two patients had auricular fibrillation (circulation times of twenty-five and twenty-eight seconds). In the 4 patients with cardiac decompensation the extremes of circulation time were from sixteen to twenty-eight seconds.

Group 4b.—Six males and 7 females, whose ages ranged from 32 to 71, were placed in this class. All of them had hypertension and some had suggestive evidence of coronary artery disease. The circulation times varied from twelve to thirty-five seconds. The patient with the shortest circulation time was able to lie perfectly flat, but she had some dyspnea on walking (very obese woman). Patients with circulation times of fourteen to seventeen seconds "had to sleep with pillows," were "short of breath at night," or had dyspnea on walking. Those with circulation times of seventeen to thirty-five seconds had definite cardiac decompensation.

Group 4c.—This division contained 11 patients, 9 males and 2 females. The ages varied from 44 to 83. They all had marked evidence of coronary artery disease. Two had auricular fibrillation, one had angina pectoris, and two had survived coronary thrombosis. The circulation times in the group ranged from eleven to forty seconds. The group with the eleven- to sixteen-second circulation time was composed as follows: 2 without symptoms (the oldest patients) gave responses at eleven and fifteen seconds; one with recent coronary thrombosis, without evidence of cardiac decompensation, at thirteen seconds; one with recently recovered cardiac compensation, at fourteen seconds; one with slight dyspnea, at sixteen seconds. The remainder obviously had cardiac decompensation and their circulation times ranged from twenty to forty seconds.

Patients with cardiac decompensation showed a definite prolongation of the circulation time. Circulation times above twenty seconds were always associated with cardiac decompensation, but one patient with unproved but probable cardiac decompensation (group 2) had a circulation time of eight seconds. On the contrary, patients with a wide assortment of diseases (one exception) did not have circulation times above sixteen seconds. This exception appears quite important and suggests that exceptions occur in both directions, namely, normal circulation time in rare cases despite cardiac decompensation and prolonged circulation time despite the absence of cardiac decompensation. These exceptions imply that this (and other similar tests) are far from infallible and cause one to doubt their application to the diagnosis of cardiac decompensation. In short, one wonders whether they are sufficiently delicate to be suggestive in early cardiac decompensation and whether they are necessary in manifest cardiac decompensation.

Nevertheless, repetitions of the test showed increased velocity of the blood stream as improvement occurred in cardiac decompensation; when the circulation time remained status quo the general situation persisted unchanged. For

example, the circulation times at two-week intervals in one individual with cardiac decompensation were thirty, twenty-two, fifteen seconds, respectively, as he improved. One patient with cardiac decompensation had a circulation time of twenty-five seconds; one and two weeks later the same reading was obtained and no recognizable change occurred in the situation during the interim. Similar results were obtained in numerous other individuals.

Volgin and Stanojević compared the circulation time obtained with lobeline with that secured by decholin and saccharin. They found the circulation time in all groups was longer with the decholin and saccharin and that variations were greater within each of the various groups, i.e., among normal individuals and among patients with cardiac decompensation. The longer time might be partly explained by the difference in the mechanism, that is, decholin and saccharin are arm-tongue methods, whereas lobeline is an arm-carotid sinus method. The results obtained by decholin and saccharin are similar, and the discrepancy in comparison to lobeline is greatest in patients with cardiac decompensation. A study of lobeline and calcium gluconate indicates that the circulation time with the former is shorter than with the latter. In ten experiments the lobeline time was one to seven seconds shorter than the calcium gluconate time in nine patients; the calcium gluconate time was shorter than the lobeline by two seconds in one patient.

A review of all the results obtained indicates that a sharp line cannot be drawn between individuals whose circulation is normal and those with cardiac decompensation by means of the circulation time. Nevertheless, several observations seem worthy of mention.

Many patients with pulmonary tuberculosis, bronchiogenic carcinoma, pleurisy, pneumothorax or anemia, exhibited dyspnea at rest or upon exertion. The circulation time was not prolonged in any of these individuals as long as cardiac decompensation was absent. Accordingly, it seems that the test may have value in assisting to differentiate between dyspneas of cardiac and pulmonary origin.

The degree of edema and the height of the venous pressure did not parallel the circulation time. In two patients who suffered from hypertension and who had marked anasarca and increased venous pressures (three plus), the circulation times were seventeen seconds in one and thirty-four seconds in the other. One patient with cirrhosis of the liver and possible beriberi heart had edema of the abdominal wall and of the lower extremities and increased venous pressure (one plus), although the circulation time was eight seconds. The circulation time does not seem to be correlated with the degree of failure of the right side of the heart, but bears a relation to left-sided decompensation. Since the occurrence of edema and the increase of venous pressure coincide with the failure of the right ventricle (other causes being excluded), the height of the venous pressure bears no relation to the circulation time. In fact, patients with normal venous pressures showed a greater circulation time than those with very high venous pressures (two and three plus). One individual with the high venous pressure (three plus) had the same prolongation of circulation time (thirty-four seconds) as another with a slight increase (one plus).

A vital capacity as low as 550 was found in a patient with a circulation time of six seconds. The patient had pulmonary fibrosis but no cardiac failure.

Another patient with a vital capacity of 3,400 and a Peabody factor of 7.5 showed a circulation time of eighteen seconds; still another individual with a vital capacity of 1,200 and a Peabody factor of 2.7 had a circulation time of eight seconds. Reductions of vital capacity not of cardiac origin do not seem to influence the circulation time.

Circulation times between three and thirteen seconds were recorded in patients with no myocardial failure. Circulation times of twenty-four seconds or more occur in persons with cardiac decompensation. Normal persons with compensated heart disease or with incipient myocardial failure had circulation times of fourteen to twenty-four seconds. The longest circulation time in a person without cardiac involvement (tonsillitis) was twenty seconds; the average for all those without cardiac disturbance was eleven seconds. The shortest circulation time in the presence of indisputable myocardial failure was thirteen seconds, the longest was forty seconds, and the average was twenty-seven seconds. Accordingly, the method cannot be employed to determine the existence of circulatory failure. However, it may assist in the differential diagnosis of dyspnea and in the determination of the therapeutic progress of a patient.

It does not seem advisable to enter into a discussion of the mechanisms thought to be involved in the lobeline test since the problem has not been completely solved. However, the weight of evidence strongly favors the participation of the carotid sinus in the response. Accordingly, the lobeline and sodium cyanide methods for the determination of the circulation time are closely allied.

SUMMARY

Lobeline was employed for the determination of the circulation time in 95 patients. The absence of intolerance, toxicity, or emutation noted by other observers found further confirmation. A new subjective response was observed; this "tickling" sensation as the end point may be employed when cough is present and might be confused with the lobeline cough; it also makes the test applicable without recourse to special devices and permits a definite result to be obtained in practically all cases.

The objectivity of the lobeline test endows it with certain definite advantages over some other substances employed for the same purpose. However, in determination of the existence of cardiac decompensation, it possesses those disadvantages which are intrinsic to any "circulation time" method. As an objective means of differentiating dyspnea of left ventricular origin and that of pulmonary origin, and as an aid in following the course of therapy, it may provide some very useful information.

REFERENCES

1. Teplov, J., and Sor, V.: *Terapevtieski Arch.* 13: 57, 1935. Cited after Stanojević.⁴
2. Volgin, O., and Stanojević, L.: *Vergleichende untersuchungen der Bilirubinämie und Kreislaufzeit bei Dekompensation des Herzens*, *Klin. Wchnschr.* 17: 569, 1938.
3. Volgin, O., Stanojević, L., and Djordjević, B.: *Određivanje brzine krvotoka lobelinom*, *Medicinski Pregled.* 11: 90, 1936.
4. Stanojević, L.: *Die Bestimmung der Kreislaufzeit mit Lobelin*, *Ztschr. f. Kreislaufforsch.* 30: 521, 1938.

5. Evzlina, M. M.: Lobeline in Determination of Velocity of Blood Circulation, *Vrach. delo.* 20: 113, 1938.
6. Trimarchi, E.: Sul metodo della lobelina per la misura della velocità circolatoria, *Riforma med.* 54: 1407, 1938.
7. Trimarchi, E.: Sull'azione farmacodinamica della lobelina, *Rassegna di fisiopat. clin. e terap.* 10: 621, 1938.

REPORT OF A CASE OF CONGENITAL ABSENCE OF THE SPLEEN AND CHRONIC AMNIOTIC FLUID PNEUMONIA

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THE congenital anomaly herein reported is not common, judging from the comparatively few such cases found in the literature. During the past ten years we were able to find only five reported cases of congenital absence of the spleen, and only one of these was in an American journal, the interesting account by M. S. Peterman in the *Journal of the American Medical Association* for October 8, 1932, of a double anomaly, the absence of both spleen and left kidney. The history of apparent constitutional inferiority that the author gave in this case is very similar to the history of the fourteen-month-old boy upon whom we performed a necropsy.

The child was resuscitated with considerable difficulty at birth, having aspirated a large amount of mucus and amniotic fluid. Examination twelve hours after delivery revealed a listless, cyanotic baby, with a weak cry and an inability to nurse, although he weighed nine pounds and was a full-term child. Coarse râles were heard over both lungs, and he seemed to breathe with considerable difficulty. The heart was normal to auscultation and percussion. At three weeks of age an x-ray examination of the chest showed marked increase in pulmonary markings, but no enlargement of the thymic shadow. The heart was normal in size, shape, and position. At this time no râles could be heard over the lungs, but the infant continued to be cyanotic; he had little appetite, and was quite feeble. When he was six months old, he developed an attack of bronchopneumonia and was acutely ill for several days. He recovered from this but continued to be marasmic and the peculiar cyanosis persisted. He was not seen again until three days prior to his death, when the mother noticed that his left foot was cold and clammy and showed a bluish discoloration. He had some fever at this time. He became rapidly worse and developed a temperature of 102.6° F., rapid respirations, and rapid pulse rate. The heart was enlarged to the left, with a heaving impulse and a blowing systolic murmur at the apex. The liver margin could be felt one and one-half fingerbreadths below the costal margin. The spleen could not be palpated. The lungs were clear. An electrocardiogram showed right axis deviation and tachycardia. The lower third of the left leg and the left foot were cold and purple, with a sharply marked border indicating beginning gangrene. No pulsation could be felt in the anterior tibial artery. Blood cultures and other indicated laboratory procedures were omitted out of deference to the parents' wishes, since it was so evident that death was imminent. Local and supportive

treatment were of no avail, and the child died the following day. Just prior to death the temperature rose to 106° F., and the pulse rate was so rapid it could not be counted.

The necropsy showed a fairly well-nourished infant. The lower third of his left foot and his left leg were gangrenous due to thrombosis of the blood vessels. The lungs appeared firmer than normal and microscopic sections showed a peculiar condition in that many of the alveoli were obliterated by fibrous connective tissue; the walls of the functioning ones were very thick, probably the result of the amniotic fluid pneumonia that the infant had at birth. The heart was greatly dilated and the pericardial sac contained about twice the normal amount of fluid which was blood-stained but not purulent. There were fresh vegetations on the mitral valves which also showed other evidence of acute inflammation. The other valves were normal. There was no evidence of any congenital anomaly of the heart which might have accounted for the cyanosis that was present throughout the child's life. A large ante-mortem clot, which was firmly adherent to the chorda tendineae and showed beginning organization, was found in the left ventricle. There were fibroblasts present as well as polymorphonuclear leucocytes and some lymphocytes. The liver was large and congested, and the liver cells showed albuminous and fatty degeneration. The kidneys showed similar changes but to a much less degree. The gastrointestinal tract and pancreas were normal. A thorough search failed to reveal a spleen, although all the viscera were removed from the abdomen. An unsuccessful attempt was made to locate a splenic anlage by following up the branches of the celiac artery, but no such vessel was present. Acute bacterial endocarditis with emboli to the blood vessels of the lower third of the left leg and the left foot and the resulting thrombosis were the immediate cause of the death of the infant. Just what part, if any, the congenital absence of the spleen could have played in the production of the marked cyanosis that persisted throughout this child's brief life is an interesting speculation. Since the spleen is such an important organ in the hematopoietic system, it is conceivable that its congenital absence might have exerted a profound and adverse influence upon the circulatory system or, for that matter, upon the whole constitutional well-being of the child.

THE PHARMACOLOGY OF SILICA*

EFFECTS AND MECHANISM OF ACTION OF FATAL INTRAVENOUS DOSES OF SILICIC ACID

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THE study presented here is part of a larger project on the nature of silicosis. The exact form of silica which produces silicosis is not known, but there is evidence that silicic acid may play a role as an intermediary chemical agent in the development of the disease.¹ We shall deal here with the results of an investigation of the pharmacology of silicic acid† and give special consideration to the following: I. Toxic and lethal dose. II. Systemic effects. III. Mechanism: (a) role of blood clot formation and (b) site of action. IV. Significance of the colloidal nature of silicic acid.

METHOD

Cats were used in our experiments. Silicic acid sol was the only form of colloidal silica examined. It was prepared by dissolving suitable amounts of sodium silicate in water and treating with an excess of hydrochloric acid. This was dialyzed against water until free of chlorides and then concentrated to somewhat more than 1 per cent by evaporation with heat under reduced pressure. The silicon dioxide content was determined gravimetrically by drying and blast burning. The solution was then diluted to 1 per cent‡ and sufficient sodium chloride was added to make an 0.85 per cent saline concentration. A small amount of hydrochloric acid was added to retard the development of gel. The solutions were stored in a refrigerator when not in use. All solutions were slightly opalescent at the outset but no gel was visible macroscopically. The pH of each batch was determined electrometrically. This varied with each specimen. The same batch of silicic acid was used in each series of experiments.

The cats were anesthetized with sodium amytal by the intravenous or the intraperitoneal route, usually the former in doses of 40 mg. per kilogram.§ In most experiments kymographic records were made of the effects of silicic acid on blood pressure, heart rate, and respiration, although some observations were made on intact unanesthetized animals. Electrocardiographic records were made

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†The terms silicic acid and colloidal silica will be used interchangeably.

‡Batches 1 and 6 were made up as 0.75 per cent and 2.0 per cent, respectively. The amount of silicic acid is always expressed in terms of silicon dioxide content.

§All doses are considered in relation to body weight, but for purposes of convenience the phrase, "per kilogram," will be omitted.

in some experiments. Silicic acid was always administered intravenously. In order to prevent the formation of intravascular clots and clots in the recording system, an anticoagulant dye was used in all but a few stated experiments. This procedure is discussed later.

All animals were examined after death. Sections of tissue were preserved for microscopic examination, but the results of this examination will form part of another report.

EXPERIMENTAL

I. Toxic and Lethal Dose.—The lethal dose of silicic acid was determined by continuous intravenous injection. The injection was stopped when blood pressure fell to about a level of 100 mm. Hg, since such falls were almost always fatal. However, more silicic acid was administered if necessary. The animals were anesthetized and the blood pressure was recorded from the carotid artery. The rate of intravenous injection was varied to determine what role the rate of administration might play in the toxicity of silicic acid.

Great variability of the fatal dose of silicic acid within each batch and between batches was a prominent feature. The average lethal dose of batches varied from 15 mg. to 193 mg. The smallest single fatal dose was 5 mg. and the largest was 284 mg. The lethal dose within each batch, however, remained in the same general range. Thus, in the batch in which 5 mg. was lethal in one case, the largest dose was 20 mg., while in the batch in which 284 mg. was the largest dose, the smallest dose was 142 mg. Lethal doses of the same specimen of silicic acid varied as much as 100 per cent on the same day, ruling out the possibility that a change in the silicic acid had occurred. The toxic dose (i.e. the dose necessary to produce a perceptible fall in blood pressure) was as variable as the lethal dose; generally it was about 60 per cent of the lethal dose.

TABLE I

TABULATION OF PH OF SPECIMENS OF SILICIC ACID AND THE LETHAL DOSE

BATCH NO.	pH	AVG. L. D. MG./KG.	NO. CATS
4	1.53	165	8
1	1.55	15	2
7	1.60	48	5
7a	1.60	53	9
2	1.78	15	3
3	1.87	30	2
6	2.40	152	10
5	2.65	193	14

The pH of the different batches of silicic acid varied between 1.53 and 2.65. Table I indicates the absence of correlation between the lethal dose and the pH. In addition, preliminary experiments with salt solutions, to which hydrochloric acid had been added to produce the same pH as that of the silicic acid specimens used in our experiments, indicated that the acidity itself was without any effect in the amounts used.

From the results in Table II it appears that the more rapid injections required larger amounts of silicic acid to produce death, but this may be due to a latent period in the development of the effects of silicic acid, so that the end

TABLE II
TABULATION OF AVERAGE RATE OF INJECTION AND THE LETHAL DOSE

BATCH NO.	AVG. RATE INJECTION MG./KG./SEC.	AVG. L. D. MG./KG.	RANGE OF RATE INJECTION MG./KG./SEC.	NO. CASES
7	0.25	48	0.19-0.37	5
3	0.26	30	0.17-0.35	2
1	0.44	15	0.33-0.54	2
2	0.53	15	0.20-0.83	3
4	1.33	165	0.00-3.91	5
5	1.40	193	0.47-2.37	14
7a	2.03	53	0.96-3.33	9
6	2.53	152	0.70-4.00	16

TABLE III
TABULATION WITHIN BATCHES OF SILICIC ACID OF THE RATE OF INJECTION AND THE LETHAL DOSE

RATE INJECTION MG./KG./SEC.	LETHAL DOSE MG./KG.
Batch 6—pH 2.40	
0.7	85
1.6	146
1.6	190
1.8	106
1.8	211
2.6	220
3.6	134
3.6	180
4.0	88
4.0	159
Batch 7—pH 1.60	
0.19	69
0.21	37
0.24	58
0.25	30
0.37	44
Batch 7a—pH 1.60	
0.96	43
1.26	63
1.28	41
1.33	56
1.54	54
2.34	82
2.92	35
3.27	49
3.33	50

Note: Batches 7 and 7a are the same specimen of silicic acid. The division into groups represents slow and rapid injections.

point was passed in the titration. In Table III, however, in which data for the lethal dose and the rate of administration for all experiments with two batches of silicic acid are given, there appears to be no significant correlation with the injection rate.

II. Systemic Effects.—The effects produced by all batches of silicic acid were qualitatively the same. In unanesthetized animals it produced an acceleration of respiration, which in the case of fatal doses was followed by progressive weakness and finally clonic convulsions. In the fatal cases the entire sequence of events usually occurred within five minutes after the injection.

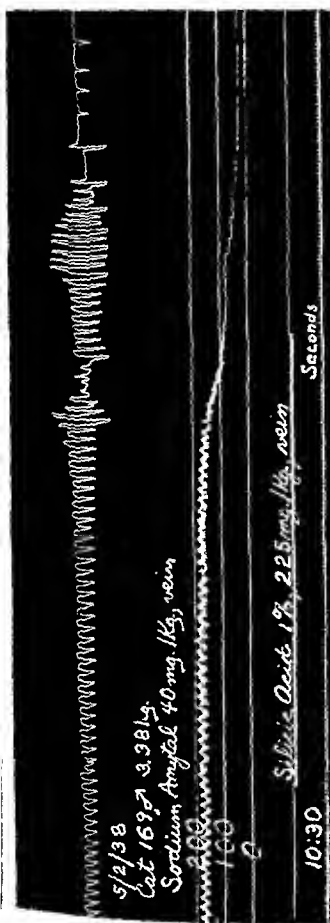


Fig. 1.—The effect of a massive intravenous dose of silicic acid on respiration, blood pressure, and heart rate. The cardiac pulsations toward the end of the tracing are too feeble to be visible in the reproduction.



Fig. 2.—Complete recovery from an insufficient dose of silicic acid, with death after an additional dose.

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2	0.53	15	0.20-0.83	3
4	1.33	165	0.06-3.91	3
5	1.40	193	0.47-2.37	4
7a	2.03	53	0.96-3.33	1
6	2.53	152	0.70-4.00	1

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Fig. 3.—Effect of silicic acid on intrapulmonary blood pressure. The first dose raises the systolic level of intrapulmonary blood pressure and causes a slight drop in systemic blood pressure. The second dose causes a rise in the diastolic level of intrapulmonary blood pressure and a sharp fall in systemic blood pressure.



Fig. 4.—Effect of sillicic acid when injected into the left auricle. The first dose administered intravenously produces a rise in both systolic and diastolic levels of intrapulmonic blood pressure, while a subsequent dose, four times as great, injected into the left auricle produces only an almost imperceptible rise in the systolic level of intrapulmonic blood pressure. The third dose injected intravenously, the same size as the first, is fatal.

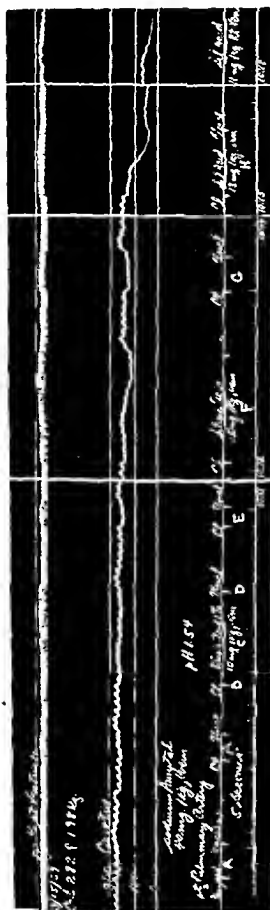


Fig. 5.—Effect of protection of lung from silicic acid. *Cl.* closing of left branch of pulmonary artery; *Uncl.*, opening of left branch of pulmonary artery; *Opn.*, opening of right branch of pulmonary artery; *C.* closing of left branch of pulmonary artery before and silicic acid is injected; *Opn.*, opening of left branch of pulmonary artery after silicic acid is injected; *A.* closing of systemic blood pressure. Opening restores to original level. *B.* closing of left pulmonary artery; *C.* injection of silicic acid with left lung out of circulation produces rise in pulmonary blood pressure and fall in systemic blood pressure; *D.* opening of left pulmonary artery. Blood pressure levels return to normal. *E.* closing of left pulmonary artery now produces greater rise in pulmonary blood pressure and fall in systemic blood pressure than in control (*A*); *F.* additional dose of silicic acid with left pulmonary artery closed produces more marked effect than before (as in *C*); *G.* now closing of pulmonary artery produces more pronounced effects on blood pressure than before, as in *A* and *E*; *H.* still more pronounced effects from another dose of silicic acid, with only slight recovery after opening of pulmonary artery.

tricle or block in the pulmonary circulation. The following observations were made in experiments designed to shed light on this question:

When silicic acid is injected intravenously, it reaches the right ventricle in relatively high concentration. It is conceivable, therefore, that the right ventricular muscle is damaged by the concentrated acid reaching it. Injections of silicic acid, therefore, were made into the left auricle, so that the acid reached the left ventricle before it circulated through the body. Such injections produced the same effects as the intravenous injections; namely, distention of the right side of the heart, rise in intrapulmonic blood pressure, and fall in systemic blood pressure. The only noticeable difference was that considerably more silicic acid was required to produce the same effects than was required with intravenous injections. Four times the dose which when injected intravenously produced marked changes in intrapulmonary pressure, was without effect when injected into the left auricle (Fig. 4).

It was also noted in a series of experiments that silicic acid was 25 per cent more toxic when the circulation of the left lung was ligated than when both lungs were intact. In another type of experiment (Fig. 5) the circulation of the left lung was constricted while silicic acid was being injected, and was re-opened after the injection had been completed. Thus the left lung was temporarily protected from the effects of silicic acid. The injection produced a rise in intrapulmonary pressure (recorded from the right ventricle) and a fall in systemic pressure. As soon as the protected lung was restored to the lesser circulation, both the intrapulmonary and systemic blood pressures tended toward normal. This protection against the effects of silicic acid was in evidence until massive (fatal) doses were given, when the protection afforded was only temporary. Control observations indicated that the constriction of the circulation to the left lung was itself without appreciable effect, whereas after the administration of silicic acid this procedure produced a definite rise in intrapulmonary and a fall in systemic blood pressures.

IV. Significance of the Colloidal Nature of Silicic Acid.—Inasmuch as silicic acid is a colloid and it has been demonstrated by Hanzlik⁴ that colloids because of their physical nature can exert systemic effects, it was decided to determine whether the colloidal nature of silicic acid played any role in the effects observed.

Cats were injected with solutions of egg albumen, agar-agar, and acacia. Sufficiently large doses of these substances produced a fall in systemic pressure and an acceleration of respiration. However, in experiments with egg albumen and acacia, in which intrapulmonary blood pressure was determined, the initial effect was a rise in systemic pressure and not a rise in intrapulmonary pressure (Fig. 6).

Egg albumen does, however, exert a synergistic action on silicic acid. An initial dose of egg albumen (300 mg.), which produced only a transient fall in systemic blood pressure, considerably increased the toxicity of silicic acid administered two to three minutes later. In 8 cats the lethal dose of silicic acid averaged 165 mg., whereas after egg albumen the lethal dose of the same specimen for 7 cats averaged only 45 mg. In another series the control lethal dose of



Fig. 6.—Effects of egg albumen and gum acacia on systemic blood pressure and pulmonary blood pressure.

silicic acid was 193 mg., and after egg albumen it was 110 mg. In both series the effects following the combination were the same as those following silicic acid alone.

DISCUSSION

The physical and chemical relationship between silicic acid and silica is very close; it has been claimed that silicic acid is hydrated silica,⁵ and x-ray diffraction observations indicate that the atomic mosaic of both is very similar if not identical.⁶ Gye and Purdy² have shown that silicic acid may, if administered over a long period of time, produce anatomical changes in organs other than the lungs, which are in many respects similar to those seen in the lungs in silicosis.

The lethal dose of silicic acid was very variable. There is no evidence that either the pH of the solutions used or the rate of administration of silicic acid played any role in the variable toxicity.

In the studies by Gye and Purdy it was demonstrated that death after intravenous injections of silicic acid was accompanied by massive intravascular clotting. These clots filled the chambers of the heart and many of the large veins. On the basis of other experiments these workers concluded that while clotting was the cause of death, it was secondary to endothelial damage. In our experiments it was demonstrated that the lethal dose and the toxic effects of silicic acid are not affected when the formation of clots is prevented by the use of an anticoagulant. It may be concluded, therefore, that while intravascular clotting may cause or contribute to the fatal outcome, there is another more direct action of silicic acid which may cause death.

The picture produced by ligation of the pulmonary artery, namely, distention of the right side of the heart, empty left side, rise in pulmonary blood pressure, and fall in systemic blood pressure, is exactly reproduced by the intravenous administration of silicic acid. The best explanation available for the production of the toxic effects of silicic acid is that it constricts the blood vessels of the lungs.

The question whether the effects of silicic acid are due to its chemical or physical nature is still an open one. Experiments by Hanzlik⁴ indicate that colloids in general may produce marked changes in systemic blood pressure, but no colloid other than silicic acid has been demonstrated to produce an initial rise in intrapulmonary blood pressure. However, the synergism between silicic acid and egg albumen is suggestive of some common action.

SUMMARY AND CONCLUSIONS

Silicic acid solutions, prepared according to a standard procedure, differed markedly in their toxicity when injected intravenously into cats. The average lethal dose of different batches varied from 15 to 193 mg. per kilogram, and was not appreciably affected by wide range in pH or rate of injection.

Immediately following injection there was a rise in intrapulmonary arterial pressure and a fall in systemic blood pressure, with an accompanying engorgement of the right side of the heart and an empty left side. Similar changes followed when silicic acid first passed through the systemic capillary bed, as was the case when injection was made directly into the left auricle.

The effects on the lung circulation were not influenced by the use of an anticoagulant, and, therefore, they were not caused by intravascular clotting. Nor is it likely, in view of the selective action on the lung capillaries and the failure of

other colloidal substances (egg albumen, agar-agar, and acacia) to produce similar changes, that they represent a nonspecific colloidal effect of silicic acid.

It is concluded that silicic acid exerts a selective action on the capillary bed of the lungs which results in an increased resistance to blood flow and accounts for the toxic symptoms and death.

REFERENCES

- Kettle, E. H.: Experimental Silicosis, *J. Indust. Hyg. & Toxicol.* 8: 491, 1926.
- Koppenhofer, G. F.: Untersuchungen zur Pathogenese silikotischer Gewebsveränderungen. IV. Mitteilung. Über die geweblichen Veränderungen nach experimenteller Zufuhr von kolloidaler Kieselsäure, *Virchows Arch. f. path. Anat.* 297: 271, 1936.
- Heffernan, P.: Some Notes on the Biophysics of Silica and the Etiology of Silicosis, *Brit. M. J.* 2: 489, 1929.
- Gye, W. E., and Purdy, W. J.: Poisonous Properties of Colloidal Silica. I. Effects of the Parenteral Administration of Large Doses, *Brit. J. Exper. Path.* 3: 75, 1922; Poisonous Properties of Colloidal Silica. II. Effects of Repeated Intravenous Injections on Rabbits; Fibrosis of the Liver, *Ibid.* 3: 86, 1922; Poisonous Properties of Colloidal Silica. III. *Ibid.* 5: 238, 1924.
- Huggett, A. StG., and Rowe, F. M.: Azo-Dyes as Anti-Coagulants, *J. Physiol., proc.* 78: 25, 1933.
- Modell, W.: Chlorazol Fast Pink BKS as an Anti-Coagulant, *Science* 89: 349, 1939.
- Hanzlik, P. J., and Karsaer, H. T.: Anaphylactoid Phenomena From the Intravenous Administration of Various Colloids, Arsenicals, and Other Agents, *J. Pharmacol. & Exper. Therap.* 14: 379, 1920; A Comparison of the Prophylactic Effects of Atropine and Epinephrine in Anaphylactic Shock and Anaphylactoid Phenomena From Various Colloids and Arsenamine, *Ibid.* 14: 425, 1920; Further Observations on Anaphylactoid Phenomena From Various Agents Injected Intravenously, *Ibid.* 23: 173, 1924.
- Hanzlik, P. J.: Basis of Allergic Phenomena, *J. A. M. A.* 82: 2001, 1924.
- Hanzlik, P. J., Do Eds, F., and Tainter, M. L.: Blood and Symptomatic Changes Following the Intravenous Administration of a Variety of Agents and Solutions, *Arch. Int. Med.* 36: 447, 1925.
- Weiser, H. B.: Inorganic Colloidal Chemistry, John Wiley & Sons, Inc., New York, 1935, Vol. 2, pages 199, 200, 226.
- Krejci, L., and Ott, E.: Study of Silica Gel (X-ray Study), *J. Physical. Chem.* 35: 2061, 1931.

TOXIC HEPATITIS DUE TO SULFANILAMIDE*

REPORT OF A FATAL CASE WITH HISTOPATHOLOGIC FINDINGS IN THE LIVER

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THAT sulfanilamide has toxic effects is generally known. That it may cause hepatitis has been stressed by several observers. Hageman and Blake¹ have observed a case of toxic hepatitis, presumably due to sulfanilamide, in an alcoholic person. Saphirstein² recorded a severe but nonfatal case of hepatitis. Long³ encountered jaundice with marked decrease in liver function on one occasion. Bannick, Brown, and Foster⁴ reported two fatal cases of hepatitis in which they believed pre-existent liver damage a possibility. Garvin⁵ recorded a series of five cases of hepatitis, one of which proved fatal, but no autopsy was obtained. Cline⁶ was the first to place on record a case of yellow atrophy of the liver with autopsy findings. Fitzgibbon and Silver,⁷ in a series of 200 cases showing toxic

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sulfanilamide effects, noted one severe nonfatal case of hepatitis. Fatal subacute atrophy of the liver with necropsy findings, however, is still sufficiently rare to warrant recording, this being only the second case to be reported in the English literature.

CASE REPORT

J. R., a white male, aged 67 years, was admitted to the surgical service of Mount Sinai Hospital on April 17, 1939, with the complaint of urinary frequency, hesitancy, and nocturia of five years' duration. He had had a similar attack ten years previous, but, following a cystoscopic examination, he seemingly improved for a period lasting five years. He had had pneumonia at the age of 12 years, and hay fever for the past four or five years. Physical examination revealed a few moist râles at both bases. His blood pressure was 180/90; examination of the abdomen gave negative findings; the prostate gland was hypertrophied, grade III. Laboratory data: phenolsulfonphthalein test was 70 per cent in three hours; blood chemistry was normal; Kline tests were negative; urine: albumin two plus; sugar (negative); blood count: innumerable red blood cells; hemoglobin 90 per cent, white blood cells 7,500. As part of preoperative treatment the patient was given on April 18, 10 grains of sulfanilamide three times a day for three days (total 100 grains) without any apparent untoward symptoms. Suprapubic prostatectomy was performed on April 22 under spinal anesthesia. Pathologic diagnosis: chronic and subacute prostatitis with nodular glandular hypertrophy and considerable hyperplasia. The patient made a good recovery and was discharged on May 10.

He was readmitted to the medical department on June 16, 1939. He stated that he felt well after leaving the hospital until about June 2, when he was given by his attending physician six 5-grain tablets of sulfanilamide daily for ten days (total of 20 Gm.) on account of pyuria. Soon after starting the drug he began to have anorexia, nausea, weakness, and began to pass "coffee-like" urine and light-colored stools. The patient blamed the medicine for his symptoms and stated that as soon as the drug was discontinued these symptoms subsided. Physical examination on this admission showed a considerable degree of jaundice, an enlarged liver palpable two fingerbreadths below the costal margin, and the edge of the spleen was palpable. There was no ascites, and there were no visible distended veins. The blood pressure was 136/84. Impression: toxic hepatitis probably due to sulfanilamide. (Dr. G., the family physician, reported that jaundice was not present on June 12, three days before admission.) Course: the jaundice increased rapidly. There was no urobilin or urobilinogen in the urine, and the stool was negative to hydrobilirubin except on July 10 and 15, when it was slightly positive. The jaundice was of the obstructive type, and it was our opinion, chiefly because of the enlarged spleen, that the patient had a toxic hepatitis merging into a chronic state, due to sulfanilamide. On July 12 signs of ascites, which progressively increased, were in evidence. On July 19 the patient was released at his own request. Laboratory examination during this admission was as follows: gastrointestinal series was negative; flat plate of the right upper quadrant was negative; electrocardiogram: P-R interval twenty-three seconds and slight slurring of QRS wave in limb leads; urine: bilirubin was positive in all specimens; urobilinogen negative; clumps of white blood cells; no leucine or tyrosine; blood: mild normocytic anemia, hemoglobin 75 to 76 per cent; white blood cells 5,700, red blood cells 3.8 million; icteric index varied between 90-67-115; van den Bergh test 19.2 units on June 17; cholesterol 210-203; esters 50 to 30; chemistry: nonprotein nitrogen 30, creatine 1.5; total plasma proteins 5.5; albumin 4.45; globulin 1.25; Kline test: diagnostic two plus, exclusion four plus on June 17 and June 19 (however, negative on first and on last admissions); stool hydrobilirubin was negative (eighteen examinations) except on July 10 and July 15, when it was slightly positive; ascitic fluid: no fixed tissue present; no cells. Pathologic report: fibrin clot. Treatment consisted of high carbohydrate diet supplemented by glucose and high vitamin diet and some bile salts, and paracentesis when indicated. Weight on admission was 163 pounds; on discharge, 163 pounds.

The patient was admitted to another service for paracentesis and peritonectomy on July 28. His diagnosis was cirrhosis of the liver.

The patient's fourth admission to the hospital and his second admission to the medical service occurred on August 4. At that time jaundice was intense, and ascites had increased. The liver edge was not felt and percussion showed it to be smaller than on the previous admission; the abdomen was distended with fluid. The course now was progressively and rapidly downward. On August 14 the patient began to get drowsy (cholemia); this drowsiness gradually merged into coma and he died on August 22, 1939.

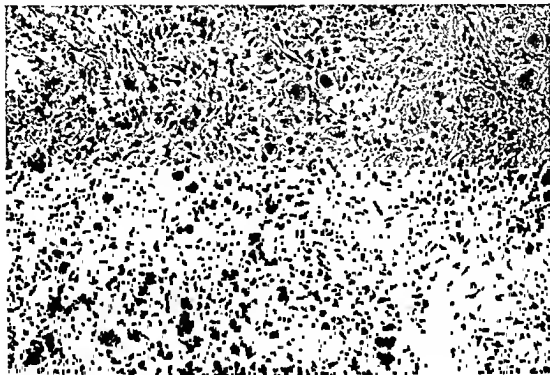


Fig. 1.—Liver. Low-power magnification. Extensive necrosis with replacement fibrosis. ("Toxic hepatitis." "Subacute yellow atrophy.")

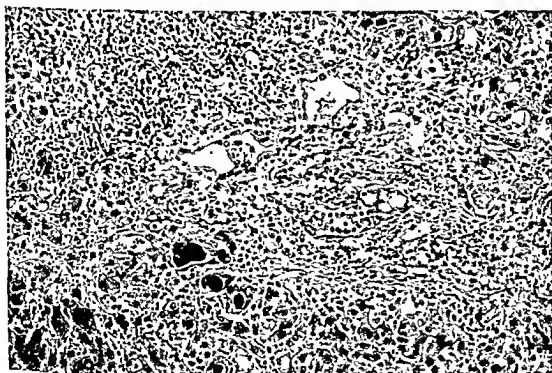


Fig. 2.—Liver. Low-power magnification. Extensive necrosis with replacement fibrosis. Beginning cirrhosis. ("Toxic cirrhosis.")

On this admission nonprotein nitrogen was 34, icteric index was 120, cholesterol 180, esters 27.5, total plasma proteins 4.65, albumin 2.32 and globulin 2.33. In the urine bilirubin was positive; urobilinogen was positive (hydrobilirubin, however, was negative on three examinations); leucine and tyrosine were negative. Blood count showed red blood cells 4 million, hemoglobin 75 per cent, white blood cells 8,000 to 12,000. Bleeding time was two minutes and clotting time was four minutes. Kline test was negative. Abdominal fluid was tapped and showed abundant growth of *Staphylococcus aureus* (contamination). The temperature

was normal throughout his hospital stay, except on admission (101.5 on June 16), on June 25 (100.5), and for a week following operation. Final diagnosis was subacute yellow atrophy of the liver and cholemin.

Permission for an autopsy was not obtained, but through a puncture wound we were able to obtain several small segments of liver tissue which form the basis of the pathological report submitted by our pathologists, Drs. B. S. Kline and A. M. Young, as follows:

Autopsy was limited. The liver is small, firm, and finely granular. On microscopic examination the liver cells in many places show early degenerative changes with some of the nuclei pyknotic and deeply blue stained, and others poorly stained or unstained with cell outlines blurred. In these areas there is abundant granular greenish-yellow bile pigment in the cytoplasm of the liver cells and homogeneous bile in the canaliculi. In other areas the liver cells are necrotic (see Fig. 1) and in many places they are absent, with apparent condensation of the stroma and bile ducts. Many of the small bile ducts are overdistended by homogeneous masses of bile. There is considerable infiltration in the periportal areas by wandering cells, with small round cells predominating (see Fig. 2). The normal lobulation of the liver is destroyed, the intact liver tissue occurring in irregular small rounded nodules characteristic of beginning cirrhosis. Anatomic diagnosis: extensive necrosis and replacement fibrosis, liver ("toxic hepatitis," "subacute yellow atrophy," and beginning cirrhosis, "toxic cirrhosis").

The patient apparently had a normal liver by the usual standards of examination, until he began to take sulfanilamide. No other drug was taken, and there is no history of previous contact with any hepatic irritants. He ingested 6.6 Gm. of sulfanilamide over a period of three days, April 18 to April 22, and again a second course of 20 Gm. over a period of ten days, June 2 to June 12. This was promptly followed by gastric upset, with nausea and vomiting, and about four days after the discontinuance of the drug he developed jaundice, enlarged liver and spleen, and later ascites. It is reasonable, if not certain, to accept a causal relationship between the drug and the hepatitis. Further, presumptive evidence may be had in the patient's own statement that gastric disturbance promptly followed the taking of the tablets, and promptly disappeared upon discontinuance of them. This case differs from the two fatal cases of hepatitis reported by Bannick, Brown, and Foster⁴ in that they believed that their patients might possibly have suffered from pre-existing liver damage; it differs also from Garvin's⁵ fatal case, in which hepatitis was complicated by exfoliative dermatitis and anemia. There were no skin lesions and no appreciable anemia in our case, and, as stated before, no evidence of previous liver damage. The Kline test was completely negative at first, then positive (diagnostic two plus, exclusion four plus), and again completely negative, and there was nothing in the history or physical examination to support the suspicion of a syphilitic involvement. Because of the age of the patient (67) and the painless progressive jaundice of the obstructive type, the possibility of malignancy suggested itself. However, the enlarged spleen, which was readily palpable on the first admission, made the differential diagnosis in favor of hepatitis and cirrhosis, and finally subacute atrophy (necrosis) of the liver. This impression was supported by a definite history of sulfanilamide ingestion, the absence of malignant cells from the ascitic fluid, the negative peritoneoscopic examination as regards tumors, the interruption in

the complete obstruction, as evidenced by the occasional finding of hydrobilirubin in the stool and urobilinogen in the urine, plus the subsequent decrease in size of the liver.

It is of interest to note that Saphirstein's² patient had an associated exfoliative dermatitis, and so did three of Garvin's⁵ series of five patients, and that the only fatality was one of these patients. Our own patient was apparently free from all skin lesions during his present illness; so was the one of Cline⁶ and of Fitzgibbon and Silver.⁷

This, then, is the second case of sulfanilamide hepatitis with post-mortem findings of the liver to be reported. With the increasing use of this drug more cases of hepatitis are to be expected. Whether this injury is due to hypersensitivity or to inherent toxicity, it is difficult to say. The case of Fitzgibbon and Silver⁷ suggests the former. After an interval of six weeks following the last dose of sulfanilamide, their patient took 15 grains (1 Gm.) more of this drug and six days later developed severe hepatitis. Similarly, in our case there was an interval of about six weeks between the two courses of the drug, and the appearance of jaundice occurred within four days after its discontinuance. Also, in 7 of 32 patients of Bannick and associates⁴ the previous administration of sulfanilamide rendered further treatment with this agent inadvisable. Recently, one of us (H. S. A.) had a similar experience with sulfapyridine. Repeating the drug after an interval of seven days brought on such severe gastric disturbance that it necessitated prompt discontinuance of the sulfapyridine, bringing prompt relief of the symptoms. Some of the patients may eventually overcome these toxic effects with the continuance of the drug, but it is a much safer procedure to discontinue it.

On the other hand, Hageman and Blake's¹ researches failed to demonstrate any precipitins for sulfanilamide in the blood serum during and following the reaction, as have attempts to demonstrate sensitivity by the patch and intradermal tests. Passive sensitization in human beings and in guinea pigs have also yielded negative results. Attempts to produce anaphylactic and skin hypersensitivity in guinea pigs have likewise failed. The prevailing view (Silver and Elliott,¹⁰ Garvin,⁵ and others) at present is that the sensitization phenomena are similar to those which occur with arsphenamine, einchophen, and aminopyrine.

There was a history of hay fever in our patient of about four to five years' duration. However, there was no opportunity for investigation of this angle, and no further enlightenment could be obtained from the family physician on this point. Its presence would have raised more than mere speculation as to the possibility of an allergic basis for the hepatitis following the second course of sulfanilamide therapy.

Apparently the dosage of sulfanilamide is not the only factor in its toxicity. Clark⁹ reports a patient who had taken 290 grains (18.85 Gm.) of sulfanilamide orally at one time "with no more serious effects than a transient decrease in the respiratory rate and a marked drowsiness, both of which symptoms were entirely relieved within twenty-four hours by forced fluids and respiratory stimulants." Cline's patient⁶ who died received 45 Gm. of sulfanil-

amide and the one of Fitzgibbon and Silver⁷ 500 grains (33.3 Gm.) of sulfanilamide. Our patient received the smallest amount, a total of 26.6 Gm. of the drug. While there is an accepted general plan of dosage, each case is an individual problem as to how much sulfanilamide it should receive. Long's studies indicate the value of a concentration of 10 to 15 mg. of the drug per 100 c.c. of blood in the fulminating cases, and from 5 to 10 mg. per 100 c.c. of the blood as a favorable concentration to obtain good results in the mild and moderate cases.

SUMMARY

We have presented a case of fatal hepatitis (subacute yellow atrophy of the liver) following the ingestion of 26.6 Gm. of sulfanilamide, and have presented the post-mortem findings of sections of the liver. This experience confirms the view of Garvin and others that sulfanilamide should be added to the list of agents which may cause severe damage to the liver, and further emphasizes the fact that it may even prove fatal. The danger of the indiscriminate use of sulfanilamide thus becomes obvious.

REFERENCES

1. Hageman, P. O., and Blake, F. G.: A Specific Febrile Reaction to Sulfanilamide: Drug Fever, *J. A. M. A.* 109: 642, 1937.
2. Saphirstein, H.: Hepatitis and Toxic Erythema With Desquamation Due to Sulfanilamide, *Urol. & Cutan. Rev.* 42: 101, 1938.
3. Long, P. H.: The Clinical Use of Sulfanilamide and Its Derivatives With Special Reference to Their Possible Toxic Effects, *Ohio State M. J.* 34: 977, 1938.
4. Bannick, E. G., Brown, A. E., and Foster, F. P.: Therapeutic Effectiveness and Toxicity of Sulfanilamide and Several Related Compounds, *J. A. M. A.* 111: 770, 1938.
5. Garvin, Curtis F.: Toxic Hepatitis Due to Sulfanilamide, *J. A. M. A.* 111: 2253, 1938.
6. Cline, E. W.: Acute Yellow Atrophy of the Liver Following Sulfanilamide Medication, *J. A. M. A.* 111: 2384, 1938.
7. Fitzgibbon, P., and Silver, B.: Necrosis of Liver (Case), *California & West. Med.* 53: 123, 1939.
8. Long, P. H., Bliss, E. A., and Feinstone, W. H.: Mode of Action and Clinical Use and Toxic Manifestations of Sulfanilamide, *J. A. M. A.* 112: 115, 1939.
9. Clark, A. L.: Sulfanilamide in Treatment of Nonspecific Infections of the Urinary Tract, *J. A. M. A.* 112: 719, 1939.
10. Silver, B., and Elliott, M.: The Use of Sulfanilamide in 1625 Cases of Gonorrhea in the Male, *J. A. M. A.* 112: 723, 1939.

TRANSIENT POSITIVE SEROLOGIC TESTS FOR SYPHILIS*

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IT IS generally recognized that syphilitic sera, upon storage, may gradually decrease in potency. As a general rule, these changes are fairly slow. It is common practice among smaller laboratories to hold positive sera for several weeks for use as controls in the performance of serologic tests.

In connection with an exchange of sera with other laboratories, we frequently noted that some which gave positive reactions in one laboratory gave negative reactions in others within a relatively short time interval. In other cases, the potency of sera remained practically unchanged over a period of time. Since the changes occurred chiefly in weakly positive sera, we attributed these changes to a decrease in reagent content. In weakly positive sera variations in sensitivity of antigens would be most pronounced.

The following event called our attention to the fact that changes may be even more pronounced. A young Mexican girl had applied for admission to a hospital for tubercular children. A blood specimen gave positive Kline, Hinton, and Kahn reactions. A second specimen gave negative reactions. A third specimen, which was divided, gave positive Kline, Hinton, and Kahn reactions in this laboratory, and negative Kahn and Wassermann reactions in another laboratory. The serum remaining from our tests was immediately referred to two other laboratories; one reported a negative Kahn reaction, the other negative Kline and Kahn reactions. The serum was returned to us, and upon re-examination gave negative Kline, Kahn, and Hinton reactions.

A fourth specimen gave negative reactions. A fifth specimen was examined separately so as to eliminate any possibility of mixing with other specimens. The specimen gave a positive Hinton, and strongly positive Kahn and Kline tests. The negative and positive controls gave negative and positive reactions, respectively. The serum and the controls were referred to two other laboratories; one reported a negative Kahn reaction, the other negative Kline and Kahn reactions. Both reported the negative control as negative and the positive control as positive. The serum was returned to this laboratory and re-examined. The serum gave negative Kline, Hinton, and Kahn reactions; the negative and positive controls gave negative and positive reactions, respectively.

The total time elapsing between our first and second examinations was less than three hours. The same lots of Kline antigen emulsion and Hinton glycerinated indicator were employed. The following day the serum was heated for ten minutes at 56° C. water bath and re-examined. The Kline, Kahn, and Hinton reactions were negative.

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As a result of this experience we decided to re-examine a number of sera on the day following examination. Our routine procedure has been to separate the sera, inactivate them for thirty minutes at 56° C. in an electrically controlled water bath, allow them to cool to room temperature, and then examine them immediately. When sera had been stored in the icobox, they were reheated for ten minutes in a 56° C. water bath before examination. In every case, the same lots of Hinton indicator and Kline antigen emulsion were used.

RESULTS

Several hundred sera have been examined in the manner just outlined. A large number of weakly reacting sera were negative upon recheck; as a general rule, strongly positive sera retained their potency over rather long periods of time. To date, we have not encountered a serum which gave negative reactions on the first test and positive reactions upon recheck. The results in Table I are representative of a large number of similar observations.

TABLE I

SPECIMEN NUMBER	EXAMINED IMMEDIATELY AFTER INACTIVATION			EXAMINED LATER			TIME BETWEEN TESTS HOURS
	KLINE	HINTON	KAHN	KLINE	HINTON	KAHN	
19236	2+	--	--	--	--	--	4
19245	3+	--	--	1+	--	--	4
19254	3+	--	--	--	--	--	24
19641	4+	+	4+	1+	+	1+	48
20180	3+	+	4+	±	±	±	24
20199	2+	+	2+	±	±	±	24
20522	2+	+	2+	--	--	--	24
20642	4+	+	4+	1+	±	1+	24
21143	2+	+	3+	2+	--	--	24
23610	2+	+	2+	--	--	--	24
25396	2+	+	1+	--	--	--	24
25441	3+	+	3+	2+	--	1+	4
26750	4+	+	4+	--	--	--	4
27880	2+	+	2+	--	--	--	4
29000	4+	+	4+	--	--	--	4

DISCUSSION

Although most of the sera which rapidly dropped in titer gave weakly positive reactions, a much larger number of weakly reacting sera continued to give weak reactions over a period of time. On the other hand, the occurrence of strongly positive reactions, which become negative within a few hours, presents a problem. At first, we felt that this might be a method of eliminating "nonspecific" reactions. During this study we have found, however, that in some cases, sera from known syphilitics might change from positive to negative, and reacting sera from individuals who were proved to be nonsyphilitic might retain their potency; in other cases, the situation might be reversed.

The simplest explanation would be that of poor performance of the laboratory tests. However, our branch laboratory at Phoenix made a very satisfactory record in the 1939 Evaluation Study, and an interlaboratory check over a long period has shown that the tests performed in the Tucson laboratory fall well within recommended limits of sensitivity and specificity.

The possibility of daily variation in sensitivity of the tests does not seem to be an adequate explanation. The same suspensions of Kline antigen emulsion and Hinton glycerinated indicator used in the first test were employed in subsequent examinations of the same serum. In a single series many specimens which gave weakly or strongly positive reactions on the first test, gave identical reactions upon re-examination at a later date. In no case did a negative serum give a positive reaction upon recheck.

SUMMARY

It was found that certain sera which gave positive Kline, Hinton, and Kahn tests, gave negative reactions when re-examined in a relatively short period of time.

AN EVALUATION OF THE COMBINED EFFECTS OF SULFAPYRIDINE AND THE BARBITURATES IN THE TREATMENT OF PNEUMONIA*

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BECAUSE of a report by Adriani¹ of New York in July, 1939, to the effect that rats placed under treatment with sulfanilamide were thereby rendered highly susceptible to the action of the barbiturates, we undertook this study. Adriani demonstrated that in rats so treated and then administered the sodium salts of methyl-butyl-ethyl-thiobarbituric acid (pentothal), isoamyl-ethyl-thiobarbiturate (thio-ethamyl), N-methyl-cyclo-hexenyl-methyl-barbituric acid (evipal), isoamyl-ethyl-barbituric acid (amytal), or methyl-butyl-ethyl-barbituric acid (nembutal), subanesthetic doses of these agents became anesthetic, and anesthetic doses of the same agents usually became lethal. The various anesthetic inhalants, such as ether and cyclopropane, on the other hand, did not have this effect on the sulfanilamide-treated rats, nor did tribrom-ethanol. The dose of sulfanilamide given the rats was from 0.5 to 1.0 mg. per gram of body weight daily for three days. Adriani concluded his report by suggesting that the combination of sulfanilamide and barbiturates may be unwise in human therapy.

We have reviewed 18 cases of primary pneumonia to determine whether the customary use of barbiturates as sedatives in conjunction with sulfapyridine had produced any recognizable deleterious effects. In one case the clinical diagnosis was pneumonia and the Neufeld reaction was positive, but the results of two roentgenologic examinations of the thorax were negative. This case was included because both sulfapyridine and pentobarbital sodium had been used during treatment of the patient.

We have presented in Table I the respective total doses of sulfapyridine or sulfanilamide and the particular barbiturate used in each case, together with the patient's age, the result of typing his sputum, and a short comment on the

*From the Mayo Clinic, Rochester.
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TABLE I
TOTAL DOSES OF ANTISEPTIC DRUGS AND BARBITURATES ADMINISTERED

PA- TIENT'S AGE	NEUFELD REACTION	DOSE OF ANTISEPTIC DRUG				DOSE OF BARBITURATE								REMARKS	
		SULFA- PYRIDINE		SULF- ANILAMIDE		PENTO- BARBITAL SODIUM		SODIUM AMYTAL		SECUNAL		PHENO- BARBITAL SODIUM		PHENO- BARBITAL	
		GR.	GM.	GR.	GM.	GR.	GM.	GR.	GM.	GR.	GM.	GR.	GM.	GR.	GM.
3 mo.	Positive	16	1.0			0.75	0.05								No ill effects from drugs; uneventful recovery
10 mo.	Negative	109.5	7.1			8.25	0.53					0.5	0.032		No ill effects from drugs; uneventful recovery
11 mo.	Negative	10.5	0.7												No ill effects from drugs; uneventful recovery
19 mo.	Positive			158	10.2	9.75	0.63					0.25	0.016		No ill effects from drugs; prolonged illness
3½ yr.	Negative	75	4.9			1.5	0.1								No ill effects from drugs; recurrence of fever
5 yr.	Positive	240	15.6			0.75	0.05								No ill effects from drugs; uneventful recovery
6 yr.	Negative	90	5.0			10.75	0.70								No ill effects; long illness; left pleural effusion
11 yr.	Negative	370	24.0			3	0.2			3	0.2	10	0.65		No ill effects from drugs; x-ray of chest negative
16 yr.	Positive	460	29.8			8.25	0.53			4.5	0.3				No ill effects; long illness; right pleural effusion
18 yr.	Negative	585	37.9					9	0.6						No ill effects; long illness; complicating parotitis
26 yr.	Negative	675	43.7												Suppurous for 18 hours after receiving sodium amytal, gr. 6 (0.4 Gm.)
27 yr.*	Positive	380	24.6												No ill effects from drugs; uneventful recovery
30 yr.	Positive	60	3.9			1.5	0.1					1.5	0.1		No ill effects from drugs; uneventful recovery
34 yr.	Negative	225	14.6			3	0.2	3	0.2						No ill effects from drugs; uneventful recovery
37 yr.	Negative	245	15.9			7.5	0.5			3	0.2				No ill effects; long illness; left pleural effusion
45 yr.	Negative	150	9.7					6	0.4						No ill effects; died of hypertensive cardiac failure; secondary cardiac arrest
70 yr.	Negative	255	16.5	160	10.1	3	0.2	3	0.2						March 1922; recovered

course of his illness and the complications, if any. It will be seen that the most frequently used barbiturate was pentobarbital sodium and that the dose of sulfapyridine in the adult patients averaged approximately 330 grains (21.4 Gm.). The ages of the patients ranged from 3 months to 92 years. The Neufeld reaction was positive for a specific type of pneumococcus in 7 of the 18 cases. In a careful analysis of the hospital records, we were unable to find any suggestion that an untoward reaction that might be attributed to a combined effect of the two types of drugs had occurred in any of the cases, save one. This was true irrespective of the age of the patient, the type of pneumonia from which he was suffering, or the presence of complicating factors, such as pleural effusion or parotitis.

The one exception involved was a woman aged 27 years, who was in her seventh month of pregnancy and who had a lobar type of pneumonia involving the left lower lobe caused by pneumococcus type XII. The day after her admission to the hospital, her temperature decreased from 103.6° F. (39.8°C.) to normal, but on the third day she began to go into labor. Six grains (0.4 Gm.) of sodium amytal and $\frac{1}{200}$ grain (0.00032 Gm.) of hyoseine were administered and she went into a deep sleep from which she could not be roused for eighteen hours. The total dosage of sulfapyridine administered up to that time was 225 grains (approximately 14.6 Gm.). The onset of labor was delayed thirteen days.

SUMMARY AND CONCLUSIONS

We have studied 18 cases in which primary lobar pneumonia or bronchopneumonia has been treated with sulfapyridine or sulfanilamide and in which the patient was given one or more of the barbiturates in sedative doses. No untoward effects were observed, except in one instance in which prolonged stupor of the patient followed the administration of 6 grains (0.4 Gm.) of sodium amytal following a previous dosage of 225 grains (approximately 14.6 Gm.) of sulfapyridine. That this reaction was the result of the combined effect of the two drugs can be postulated, but the postulation would be difficult to prove.

It may be concluded, therefore, that the barbiturates in ordinary sedative doses may be used with apparent immunity for patients receiving sulfapyridine or sulfanilamide.

REFERENCE

1. Adriani, John: Effects of Anesthetic Drugs Upon Rats Treated With Sulfanilamide, J. LAB. & CLIN. MED. 24: 1066, 1939.

CLEARANCE TESTS IN RELATION TO RENAL PATHOLOGY*

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FOR some time we have been studying the effects of various anesthetics on kidney function. In this report the results of 275 urea clearance tests and 106 creatinine clearance tests on 10 dogs are presented. The following range of control values of urea clearance per square meter per minute has been noted: Orth,¹ 34.9 c.c.; Rhoads,² 49.8; and Ralli,³ 99.5. Because of this great variation and the inconstant results in some of our dogs under anesthesia, it seemed advisable to do autopsies at the end of our study. We are deeply indebted to Professor Helen Ingleby and her staff for autopsy reports on 7 of our dogs. Although complete autopsies were performed, only the kidney findings will be included in this paper.

On each experimental day, body weight, blood pressure (Allen⁴), respiratory rate, heart rate, rectal temperature, erythrocyte and leucocyte counts, and hemoglobin (Newcomer method) were determined. The average control values for each dog are shown in Table I. Urea clearances were done by the Van Slyke⁵ hypobromite method; creatinine clearances were done by the Holten and Rehberg⁶ method. Adult female dogs, in reasonably good health and well trained, were used. They were allowed to lie comfortably between catheterizations on the laboratory floor on blankets. Tests were done eighteen to twenty-one hours after the last meal, and no more than two tests were done on one dog in one week. The dogs were permitted to exercise daily on the roof. Some of the dogs were studied for as long as sixteen months before coming to autopsy.

Some of the dogs were fed Red Heart C, some Victory Dog Food, and the remainder Cero-Meato. Three dogs were studied for several months on each of the diets in turn, and no significant changes in urea clearance were found. We noticed that all the dogs gained weight on Cero-Meato; the pathologist stated that the dogs which had been fed Cero-Meato seemed to be in better general condition at the time of autopsy than the dogs which had been fed either of the other diets.

A brief report of the results of each anesthetic will be given. In each anesthetic period surgical anesthesia was maintained for one hour. The clearance tests on the day after the anesthesia were compared with those before and with those one, two, and more weeks after.

*From the Department of Physiology, Woman's Medical College of Pennsylvania, Philadelphia.

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TABLE I

1. Number of tests	6. Heart rate	10. Range—urea clearance	13. R.B.C. count (millions)
2. Diet	7. Blood urea nitrogen	11. Creatinine clearance	14. W.B.C. count (thousands)
3. Rectal temperature	8. Blood creatinine	12. Hemoglobin (Gm.)	
4. Respiratory rate	9. Urea clearance		
5. Blood pressure			

AVERAGE OF CONTROL VALUES DURING STANDARDIZATION

DOG	R	N	W	C	D	K	L	M	F	B
1	4	4	12	5	4	5	4	6	4	8
2	Red Heart C	Red Heart C	Red Heart C	Victory	Victory	Cero-Meato	Cero-Meato	Cero-Meato	Victory	Cero-Meato
3	100.4	100.8	101.7	100.7	100.9	100.7	102.2	100.4	101.4	101.4
4	17	24	45	25	28	26	18	17	26	18
5	162/72	220/111	164/83	160/60	168/69	167/60	161/56	167/60	157/67	166/60
6	135*	116*	114*	86	82	89	96	85	83	95
7	12.0	17.2	12.3	11.2	11.7	14.6	12.0	14.4	9.3	11.9
8	—	—	—	—	—	9.64	8.09	10.35	—	—
9	56.2	54.9	55.5	61.0	40.5	64.3	71.3	59.5	59.9	64.5
10	52.9	52.0	47.5	53.8	32.8	54.2	51.1	55.3	56.8	35.7
	57.8	58.8	69.9	67.4	44.8	88.0	110.9	68.5	61.4	132.3
11	—	—	—	—	—	75.8	74.3	77.1	—	—
12	11.57	13.41	12.21	10.92	11.88	10.60	11.12	11.10	10.34	11.00
13	5.75	6.63	6.62	6.07	7.41	7.28	7.38	7.82	5.46	6.35
14	5.4	8.6	10.0	12.1	9.8	17.0	10.2	14.6	9.2	12.0

*The heart rate was determined from the electrocardiogram instead of by stethoscope.

(a) Cyclopropane* (Diet—Red Heart C)

1. Dog R. During eight months cyclopropane was administered ten times, and the dog seemed in excellent health throughout. The most significant finding was the increase of about 40 per cent in the urea clearance on the day after the anesthesia; this was noted each time cyclopropane was administered. The average control value was 56.2 c.c. per square meter per minute, as shown in Table I, and the average value for the day after anesthesia was 79.5; this finding does not agree with that of Orth.¹ The urea clearance returned to the control value within one week after the anesthesia.

After 42 clearance tests and ten administrations of cyclopropane this dog was brought to autopsy by a lethal dose of the same anesthetic. The kidneys showed congestion only; consequently, we feel that these results are typical of cyclopropane.

2. Dog N. It may be noted in Table I that this dog showed a higher blood urea nitrogen than any of the others, and it was the only one with hypertension. It showed a rather fixed urea clearance in the 11 tests performed. Cyclopropane was administered only three times, since the dog died just at the end of the third period. The control value for urea clearance was 54.9; after the anesthesia it was 54.3.

At autopsy the kidneys showed capillary engorgement of the glomeruli and cloudy swelling of the tubules. This pathology, together with the hypertension and higher blood urea nitrogen, probably was a determining factor in the fixed clearance.

3. Dog W. Thirteen clearance tests were done on this dog. The average control value was 55.5; after one administration of cyclopropane it increased

*We are greatly indebted to the Foregger Company for the generous loan of the Foregger instrument, Metric Model No. 4, for administration of cyclopropane.

to 66.7, or about 20 per cent. Since this dog was not able to be trained to our satisfaction, it was returned to the kennels.

(b) Pentobarbital sodium (Abbott) (Diet—Victory Dog Food)

Twenty-two anesthetic periods were studied on Dogs C, D, and F. The following results were found for urea clearance:

DOG	CONTROL	AFTER ANESTHESIA
C	61.0	55.2
D	40.5	44.7
F	59.9	69.0

Since comparable results were found at some time during the establishing of the control values in two of the dogs, we felt that there were no significant changes which could be attributed to the anesthesia.

(c) Sedative dose of pentobarbital sodium followed by ether, drop method, for one hour (Diet—Red Heart C)

Dogs C, D, and F were left in the kennels for the summer and were studied again in the autumn. After new controls were established, each dog was given ether once. The following results were found:

DOG	NEW CONTROL	AFTER ANESTHESIA
C	56.7	72.2
D	47.0	49.9
F	64.8	86.7

The urea clearance increased markedly in two and only slightly in one the day after ether was administered. Orth¹ reported practically no change after ether. The urea clearance tended to return to the control value within one week.

The diet was changed to Cero-Meato at this time. After allowing about one month for complete recovery from ether and for adjustment to the new diet, new controls were established and additional studies were made. Creatinine clearances were begun and the two clearance tests were done simultaneously. According to Jolliffe,⁸ the administration of creatinine does not affect the rate of urea excretion.

(d) Sodium amytal (Diet—Cero-Meato)

Eighteen periods of anesthesia on Dogs C, D, K, and L were observed. The following results were found:

DOG	UREA CLEARANCE		CREATININE CLEARANCE	
	CONTROL	AFTER ANESTHESIA	CONTROL	AFTER ANESTHESIA
C	68.9	68.9	75.9	91.3
D	50.4	62.5	59.5	69.3
K	64.3	72.9	75.8	107.8
L	71.3	49.8	74.3	86.7

As noted, the urea clearance did not show a constant change. The creatinine clearance increased in every case. The autopsy reports on these dogs are instructive.

1. Dog C was brought to autopsy after having had 52 urea clearance and 20 creatinine clearance tests. The glomeruli of the left kidney showed a thickening of the basement membranes of the capillaries, Bowman's capsules, and afferent arterioles. Many of these arterioles were dilated and the walls thickened where they entered the glomerular tuft. The basement membrane of the tubules was slightly thickened. There were thin-walled spaces lined by endothelium and containing blood scattered throughout the kidney substance and pelvis.

The right kidney showed intense congestion and hemorrhage. There was a triangular area infiltrated with mononuclear cells beneath the capsule. This area was partly fibrosed. The afferent arterioles and basement membranes were less affected than in the left kidney, but inflammatory lesions were more prominent. The diagnosis was interstitial nephritis and nephrosclerosis.

This dog showed relatively high clearances in spite of the renal pathology, but the variations after anesthesia were less marked than in some of the other dogs.

2. Dog D was brought to autopsy after 54 urea clearance and 27 creatinine clearance tests. The only report on the kidneys recorded by the pathologist was that the glomeruli were intensely congested and there was slight cloudy swelling of the tubules.

3. Dog K was brought to autopsy after 19 urea clearance and 18 creatinine clearance tests. The blood vessels in the interstitial tissue and some glomeruli were congested. There was a slight thickening of the basement membrane of the capillaries of the glomeruli and also a thickening and dilatation of some of the afferent arterioles. Many Bowman's capsules were thickened, and in some areas the capsules were enormously distended with granular material. These glomeruli resembled those seen in lipid nephrosis in human kidneys. Cloudy swelling of the convoluted tubules was noted.

This dog had a high white blood cell count, as shown in Table I, and gave very irregular clearance tests, as might be expected in view of the renal pathology.

4. Dog L was brought to autopsy after 7 urea clearance and 7 creatinine clearance tests. The kidneys were intensely congested. The glomerular capillaries were enormously distended, with an occasional hemorrhage into the capsule. The appearance of some of the glomeruli suggested a hemorrhagic nephritis. The clearances were irregular in this dog.

(e) Sodium barbital (Diet—Cero-Meato)

Dogs M and F were used, and only four periods of anesthesia were observed. The following results were found:

DOG	UREA CLEARANCE		CREATININE CLEARANCE	
	CONTROL	AFTER ANESTHESIA	CONTROL	AFTER ANESTHESIA
M	59.5	61.8	77.1	53.1
F	60.6	46.5	58.8	85.5

As noted, the results were very irregular. Dog M, after 10 clearance tests, was used for other purposes and did not come to autopsy.

Dog F had been used for 59 urea clearance and 24 creatinine clearance tests before coming to autopsy. The kidneys showed congestion. In many glomeruli there was extreme dilatation of the afferent arterioles and the capillaries. Hemorrhage was evident in some of Bowman's capsules.

Dog B was used only for 8 control urea clearance tests. Since the range was from 35.7 to 132.3, it was used for other purposes and was not brought to autopsy.

SUMMARY AND CONCLUSIONS

1. The urea clearance increased as much as 40 per cent on the day following the administration of cyclopropane and returned to normal within a week in a dog with no renal pathology. In a dog with hypertension, high blood urea nitrogen, and some renal pathology, a fixed urea clearance was found.

2. The administration of pentobarbital sodium (Abbott) failed to produce greater changes in the urea clearance than were found in the normal range of two of the dogs.

3. The administration of ether (drop method) after a sedative dose of pentobarbital sodium was followed by a marked increase in the urea clearance in two dogs and a very slight increase in a third dog.

4. The administration of sodium amytal was followed by an increase in the urea clearance in one dog with little renal pathology; the results were inconstant in three dogs with considerable renal pathology. The creatinine clearance increased in every case; there was great variation in the amount of increase.

5. The administration of sodium barbital was followed by a decrease in the urea clearance and an increase in the creatinine clearance in one dog with some renal pathology.

6. High leucocyte counts, elevation of rectal temperature, and renal pathology seem to be associated with inconstant urea clearance.

7. The inconstancy of urea clearance results has been noted by Goldring,⁸ Holman,¹⁰ and Van Slyke.¹¹ We consider it worth while to follow a series of clearance tests on one animal by autopsy.

8. It is possible that sedation with barbiturates on the night preceding a clearance test may influence the results. An elevation of temperature and a high leucocyte count may likewise affect the results.

REFERENCES

1. Orth, O. S., and Stutzman, J. W.: Constancy of Urea Clearances in Dogs Following Surgical Anesthesias With Cyclopropane, Ether and Chloroform, *Proc. Soc. Exper. Biol. & Med.* 39: 403, 1938.
2. Rhoads, C. P., Alving, A., Hiller, A., and Van Slyke, D. D.: The Functional Effect of Explanting One Kidney and Removing the Other, *Am. J. Physiol.* 109: 329, 1934.
3. Ralli, E. P., Brown, M., and Pariente, A.: The Urea Clearance Test in Normal Dogs, *Am. J. Physiol.* 97: 432, 1931.
4. Allen, F. M.: Auscultatory Estimation of Blood Pressure in Dogs, *J. M. Research* 4: 431, 1923.
5. Van Slyke, D. D., and Kugel, V. H.: Improvements in Manometric Micro-Kjeldahl and Blood Urea Methods, *J. Biol. Chem.* 102: 489, 1933.
6. Holten, C., and Rehberg, P. B.: Studies on Pathological Function of Kidneys in Renal Disease, Especially Bright's Disease, *Acta Med. Scandinav.* 74: 479, 1931.
7. Hafkesbring, R., Greisheimer, E., and Magalhaes, H.: Effects of Repeated Anesthetic Doses of Barbiturates, *J. Pharmacol. & Exper. Therap.* 66: 95, 1939.

3. Jolliffe, N., and Smith, H. W.: The Excretion of Urine in the Dog. I. The Urea and Creatinine Clearances on a Mixed Diet, *Am. J. Physiol.* 98: 572, 1931.
4. Goldring, W.: Studies of the Kidney in Acute Infection. II. Observations With the Urea Clearance Test in Acute Rheumatic Infection, *J. Clin. Investigation* 10: 345, 1931.
5. Holman, R. L.: Observations on the Urea Clearance in Dogs, *Am. J. Physiol.* 104: 615, 1933.
6. Van Slyke, D. D., Rhoads, C. P., Hiller, A., and Alving, A.: Relationships Between Urea Excretion, Renal Blood Flow, Renal Oxygen Consumption and Diuresis. The Mechanism of Urea Excretion, *Am. J. Physiol.* 109: 336, 1934.

INCIDENCE OF BACTERIEMIA IN PATIENTS WITH DENTAL DISEASE*

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FROM clinical observation one is aware of the fact that bacteriemia must occur frequently without peripheral manifestations of sufficient grade to say the patient is "diseased." Proof may be seen in the fact that traumatized tissues in which abrasion of the skin was absent are frequently infected. Thus hematomas that come up infected or the osteomyelitis that follow slight trauma may be taken as good evidence that a bacteriemia existed and that an infecting organism reached the point of infection through the blood stream. As promulgated by Rosenow¹ and Fischer,² the teeth act as chronic foci, are a common source of invasion of the blood stream, and hence are responsible for the diseased processes that occur in distant lying tissues.

If the view of these authors is correct, one should expect to be able to uncover the existence of bacteriemias in patients affected with chronic dental disease. Okell and Elliot³ found a transitory bacteriemia in from 60 to 70 per cent of their patients after the extraction of teeth under general anesthesia; Burket and Burn,⁴ in 17 per cent of their patients when local anesthesia only was employed. The latter explained their low incidence by saying that the suprarenin employed acted as a vasoconstrictor to render the vessels less permeable to the entrance of microorganisms.

To learn how frequently bacteriemia occurs in dentally affected patients not subjected to operation, the following experiment was performed: Three hundred and thirty-six patients with varying degrees of dental caries and pyorrhea were asked to chew a 1 inch cube of high melting point paraffin. After chewing this for one-half hour, blood cultures were taken from the femoral artery.⁵ (The area was scrubbed with tincture of green soap and water, followed by iodine and alcohol, and then the application of tincture of merthiolate. Cultures from skin areas so treated were routinely negative.) The blood withdrawn from the artery was mixed with brain broth containing one-half of 1

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per cent peptone, and allowed to stand for six days at 37° C. Thereafter subcultures were prepared in heart infusion agar.

These blood cultures proved positive immediately after chewing in 153 or 55 per cent, of the patients studied. Follow-up blood cultures were not taken to determine how long such a bacteremia might persist.

The organisms found were a small diplococcus in 84 per cent of the positive cultures and a slowly growing *Staphylococcus albus* in the remaining 16 per cent. One patient displayed both organisms. Subclassification was not completed.

SUMMARY

Of 336 patients with dental disease, 185 gave positive arterial blood cultures after they had chewed paraffin for one-half hour.

REFERENCES

1. Rosenow, E. C.: Elective Localization of Streptococci, J. A. M. A. 65: 1657, 1915.
2. Fischer, Martin H.: Personal communication.
3. Okell, C. C., and Elliot, S. D.: Bacteremia and Oral Sepsis With Special Reference to the Etiology of Subacute Endocarditis. Bacteremia Following Dental Extractions, Lancet 2: 869, 1935.
4. Burket, Lester W., and Burn, Casper G.: Bacteremias Following Dental Extractions, J. Dent. Research 16: 521, 1937.
5. Murray, Maynard, and Moosnick, Frank: Simultaneous Arterial and Venous Blood Cultures, J. LAB. & CLIN. MED. 26: 382, 1940.

THE HISTAMINE TREATMENT OF ALLERGIC DISEASES*

I. ASTHMA AND VASOMOTOR RHINITIS

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NUMEROUS observations have led to the assumption that the symptoms of experimental anaphylaxis and of clinical allergy are caused by histamine or a histamine-like substance. Dale's¹ theory of anaphylaxis presupposes that the combining of antigen and antibody in sensitized tissues leads to cell lesion and the liberation of preformed histamine or of a histamine-like substance. Lewis and his collaborators Grant and Hare^{2, 3} expanded this theory to explain the "anaphylactic" skin reactions in allergic human beings. In certain individuals heat, cold, or mechanical irritation, in degrees perfectly tolerated by normal persons, causes cell lesion and the liberation of histamine from the tissues (Duke, Bray, Horton⁴). The clinical manifestations of these "physical" allergies are usually the same as those in the "atopic" allergies.

Fuehner⁵ was the first to show that it is possible to induce refractoriness to histamine in normal animals. This phenomenon was observed in normal human beings by Lewis and Grant.⁶ Hare showed in allergic persons that "the histamine stimulus renders the skin refractory to the anaphylactic poison."

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Nonspecific "desensitization" with histamine was achieved by Farmer,⁷ who was able to induce diminished sensitiveness to serum in serum-sensitized guinea pigs through oral and parenteral application of histamine.

The theoretical and practical implications of these observations for the understanding and treatment of allergic diseases are obvious. The histamine theory would explain why the symptoms of allergic conditions are independent of the nature of the causative allergen, and dependent only on the reaction of the shock organs. The possibility of achieving refractoriness to histamine would reduce the treatment of allergic diseases to a common denominator and thereby make for much simplification. It would, furthermore, be very useful in all those cases of multiple sensitiveness in which several allergens would have to be used for specific desensitization, and also in those cases in which the causative allergen or allergens could not be discovered.

Histamine was first used in the treatment of an allergic condition by Ramirez and St. George⁸ in 1924. These authors had observed a group of patients with what they termed "endogenous" asthma. The patients who did not as a rule react to the various proteins tested⁹ but gave "an exceptionally strong reaction with solutions of histamine," were suffering, according to Ramirez and St. George, from "histamine sensitivity." Ten patients were treated with subcutaneous injections of histamine in doses ranging from 30 to 750 gamma; very good results were achieved in two patients.

The cases of Ramirez and St. George fit neither into the category of "atopic" nor into "physical" allergy. Recently Horton⁹ has described a syndrome, which he calls "erythromelalgia of the head" (vascular headache), and which he attributes to histamine. Relief of the symptoms in these cases was also achieved by histamine injections. It is possible that the cases of Horton belong to the same category as those of Ramirez and St. George.

In 1927 Friedlaender and Petow¹⁰ reported that they had used histamine in the treatment of migraine of various origins. Ernestene and Banks,¹¹ Gajdos,¹² and Joltrain¹³ treated urticaria with histamine; and Collens and his collaborators¹⁴ used it successfully in a diabetic person who had developed insulin sensitivity.

The reports on the use of histamine in bronchial asthma are few and far apart. In 1932 Stahl and Masson¹⁵ in Strasbourg, and two years later Piquet,¹⁶ Stahl's pupil, treated asthmatic attacks by applying histamine to skin scarifications. In 1935 Thiberge¹⁷ in this country, and Dszinich¹⁸ in Hungary reported on the treatment of asthma with injections of histamine. Thiberge used large doses of histamine: 0.5 c.c. of a 1:1,000 solution per injection. Dszinich used much smaller amounts, his initial doses in severe cases being 0.01 gamma, and in milder cases 0.1 gamma (0.00001 mg. and 0.0001 mg., respectively). Jacquelin¹⁹ in 1937 stated that he had applied histamine by iontophoresis in a few cases of asthma. Openchowski²⁰ mentioned having used histamine in asthma and other allergic conditions, but he does not give any details. All these authors were impressed by the results they achieved.

In view of these favorable clinical reports and of the afore-mentioned theoretical considerations, we felt that the use of histamine in allergic diseases

CASE	AGE	DIAGNOSIS	YEARS OF ILLNESS	SEVERITY	RESULT OF SKIN TESTS	TREATMENT				RESULT
						1ST IN-JECTION	HIGHEST DOSE	TOTAL IN-JECTIONS	DURATION OF TREATMENT	
1. J. F. ♂	48	Asthma Rhinitis	36	Moderately severe	Milk ++ Feathers + Sheep wool +	0.1	30.0	31	12/16/38-5/12/39	Marked improvement
2. J. C. ♂	30	Asthma	4	Moderately severe	Negative	0.1	15.0	24	12/7/38-5/12/39	Marked improvement
3. F. L. ♂ (Baker)	36	Rhinitis	3	Severe	Wheat +++	0.1	40.0	24	2/21/38-7/6/38	Very marked improvement
4. J. H. ♂	8	Asthma Rhinitis Eczema	4½ 7½	Moderately Severe	Rabbit dander + ?	0.01 0.1	2.5 0.8	17 8	3/30/38-7/1/38 10/3/38-12/9/38	Very marked improvement
5. J. M. ♂	6	Asthma	2	Severe	House dust ++ Dog dander ++	0.01 0.01	0.1 0.5	7 21	11/5/37-11/24/37 3/9/38-6/22/38	No improvement
6. M. M. ♀	28	Asthma	5	Severe	Negative	0.1 0.1	0.75 6.0	5 14	11/29/37-12/17/38 10/3/38-8/12/38	No improvement
7. I. M. ♀	33	Asthma Rhinitis	5	Moderately severe	House dust ++ Dog dander ++	0.05	25.0	34	4/22/38-9/9/38	Marked improvement
8. A. O. ♂	11	Asthma	6	Moderately severe	House dust ++ Sheep wool ++ Horse dander + Orris root +	0.05	10.0	15	11/29/37-1/5/38	Very marked improvement
9. W. K. ♂	10	Asthma	4	Severe	House dust ++ Sheep wool ++ Feathers + Carrot + Lettuce +	0.02 0.02	1.0 0.1	16 19	1/30/39-3/27/39 4/24/39-6/21/39	No improvement
10. A. F. ♀	43	Asthma Rhinitis	2	Moderately severe	Negative	0.1	25.0	18	9/7/38-11/7/38	No improvement
11. B. S. ♀	41	Asthma Rhinitis	1	Severe	House dust + Feathers + Monilia ++ Ragweed ±	0.1 0.01	0.3 0.15	13 28	12/28/38-2/3/39 2/24/39-7/31/39	No improvement (condition worse) Improvement
12. H. J. ♂	33	Asthma Eczema	30	Moderately severe	House dust ++ Sheep wool + Feathers + Rabbit dander +	0.1 0.1	15.0 1.5 20.0	34 9 20	11/5/37-7/1/38 12/5/38-1/18/39 5/24/39-8/16/39	Very marked improvement

TABLE I—CONT'D

Pt. R. H. S.	Sex	Age	Asthma	1	Moderately severe	Cottonseed + + +	0.01	0.5	30	10/11/37- 7/13/38	Marked improvement
14. M. A.	♀	29	Rhinitis Eczema Hay fever	6 4 2	Moderately severe	House dust + Orchis root + Ragweed + Peanut + + Bean + Potato + Tomato + Mustard +	0.1	3.5	13	1/31/38- 3/11/38	No improvement
15. M. R.	♀	21	Rhinitis	2	Moderately severe	Corment + Tea leaf + + Orange + +	0.1	10.0	26	5/ 5/37- 7/26/37	Marked improvement
16. E. S. (Baker)	♂	36	Asthma Rhinitis	2	Moderately severe	House dust + + Feathers + + Sheep wool + + Wheat + + +	0.1	5.0	17	1/26/38- 4/27/38	Marked improvement
17. K. F.	♀	54	Asthma	14	Severe	Orchis root + + + Cat dander + (+) Dog dander +	0.01 0.02	4.5 10.0	44 29	1/19/38- 7/ 6/38 9/27/38- 6/21/39	Improvement
18. H. V.	♂	7	Asthma Rhinitis	11 1/2 3	Moderately severe	House dust + + Feathers + + + Rabbit dander +	0.01	1.0	28	1/20/39- 7/24/39	Very marked improvement
19. J. B.	♀	6	Asthma Hay fever	1	Moderately severe	Dog dander + (+) Rabbit dander + + Feathers + + Knopok + (+) Ragweed + +	0.01 0.01	1.6 0.7	15 12	11/ 4/38- 1/ 9/39 5/15/39- 7/10/39	Very marked improvement
20. M. G.	♀	27	Asthma	2	Severe	Negative	0.01	0.6	14	2/ 8/39- 4/17/39	Very marked improvement
21. E. J.	♂	68	Asthma	50	Severe	Tobacco + Feathers + +	0.1	20.0	19	12/28/38- 1/28/39	Improvement
22. J. D.	♂	57	Asthma	8 mo.	Severe	Negative	0.05 0.1	20.0 20.0	37 28	12/16/37- 8/ 3/38 10/11/38- 6/13/39	Marked improvement
23. H. W.	♀	50	Asthma	30	Severe	Negative	0.01	3.0	20	1/ 9/39- 6/ 2/39	Very marked improvement

could be of essential value. It was necessary to investigate further the question of dosage and of appropriate mode of application. We felt that injection would be preferable to scarification or iontophoresis, as neither of these latter methods allows accurate dosage. However, we believed that the smaller amounts of histamine which were used in these methods, and which were also recommended by Dzsinič, would give better therapeutic results than the larger doses used by the earlier workers.

We have been using subcutaneous injections of histamine phosphate* in the treatment of various allergic diseases for three years. No hard and fast schedule of dosage has been developed. Following the suggestion of Dzsinič, the initial dose in mild cases of asthma is 0.1 gamma, and in more severe cases it is 0.01 gamma. The injections are increased each time by 50 per cent, if well tolerated, and in the beginning they are given two to three times weekly. They are later spaced at approximately five-, seven-, ten-, fourteen-, and twenty-one-day intervals. The rapidity with which the dose is increased and the spacing of the injections will depend upon the patient's tolerance and the results achieved. The maximum dose has been 50 to 75 gamma. However, in most instances very satisfactory therapeutic results have been achieved with much smaller amounts of histamine. If an injection is not well tolerated, or if a marked local reaction occurs, a smaller amount should be given the next time. In some instances, after marked improvement had been achieved in the course of treatment, the patient's condition gradually became worse. Since we felt that the patients were possibly getting too much histamine, we decreased the amount injected, and the patients again improved. In a number of cases even such small amounts of histamine as 0.03 to 0.07 gamma have caused marked exacerbation of the asthmatic attacks. It was necessary to reduce the injections to 0.005 gamma and to increase the doses very slowly. There is no method of ascertaining the amount of histamine needed in each individual case other than following the clinical course.

As in specific desensitization, histamine injections should be continued over a considerable period of time, especially if the therapeutic results are good. We have found it advisable to give a second or third series of injections after a pause of several months, even in the absence of symptoms.

We have given several thousand individual injections of histamine and have encountered a systemic reaction in only two instances. In the one case, after the injection of 1.0 gamma, the patient who was suffering from vasomotor rhinitis developed severe urticaria, which started at the site of the injection and spread over the entire body. The urticaria persisted for several days. In the other case the patient developed angioneurotic edema of the eyelids on several occasions after the injection of small amounts of histamine.

The precautions to be taken are the same as in specific desensitization. The injections should not be given into a venule or capillary, adrenalin and a tourniquet should always be on hand, and the patient should be observed for ten to fifteen minutes after the injection. However, we believe that histamine treatment causes systemic reactions less frequently than specific desensitization.

*Parke-Davis & Co. product.

We have treated with histamine 54 patients suffering from asthma, vasomotor rhinitis, or both, whose symptoms were in most instances due to allergens other than pollens. (We intend reporting on the pollen-sensitive patients in another paper.) Table I contains data on only 23 of the 54 patients. These patients were observed sufficiently long to allow conclusive evaluation of the effectiveness of histamine treatment.

All patients received physical and x-ray examinations, a routine urine examination, and a complete blood count; skin tests were carried out with the most important foodstuffs and inhalants. In almost all cases the patient was also examined otolaryngologically. As shown in Table I, the majority of the patients gave positive skin reactions to one or more allergens. These allergens were eliminated if possible, and the patient received histamine if the elimination did not lead to the desired therapeutic result.

CASE REPORTS

The following three case histories, which we present in detail, are illustrative of successfully treated patients:

CASE 3.—F. L. is a 36-year-old baker who has been suffering from severe vasomotor rhinitis for three years. His attacks of paroxysmal sneezing and profuse watery nasal discharge come on only in the bakeshop and only when working with wheat flour. They commence after he has been in the bakeshop for one to two hours, and last for many hours. His personal and familial histories are not contributory. Two years before he visited us he had a tonsillectomy; one and one-half year before, a resection of nasal septum with some improvement. X-ray of the chest and blood count were normal. Intradermal skin test with wheat extract was strongly positive. Histamine treatment was started with 0.1 gamma on Feb. 21, 1938. Decided improvement was noted after the eleventh injection, 3.0 gamma, on March 21. Improvement continued and the patient was able to work during April, May, and June without any symptoms of rhinitis. He was receiving injections of histamine during this time. The last injection of 40 gamma was given on July 6. The patient came back to the clinic on Sept. 21, 1938. He had been working during the summer and had had only infrequent, very mild symptoms. During the week prior to his readmission he had been sneezing more frequently. He was given four injections of histamine, and then did not return to the clinic as he was again feeling better. On July 28, 1939, he reported that he had been steadily working with wheat flour since Nov. 1, 1938, and that he had had no symptoms of rhinitis.

CASE 18.—H. V., a 7-year-old boy, has been suffering from rhinitis for three years and from bronchial asthma for one and a half years. His first attack of asthma occurred two weeks after tonsillectomy and adenoidectomy. The attacks come during the day and night, without any seasonal incidence. His mother has had eczema for eight years. Physical examination was negative; x-ray examination of the chest revealed slightly emphysematous lungs; differential blood count: 7 per cent eosinophiles; skin tests: house dust ++, feathers +++, rabbit dander +. Elimination of feathers from the child's environment brought no relief of symptoms. On Jan. 20, 1939, he was given his first injection of histamine, a 0.01 gamma dose. The allergic symptoms became milder soon after the beginning of treatment. Coughing and sneezing persisted longer than the asthmatic attacks. In April the patient had an attack of asthma which lasted for three days. At that time he was given 15 injections of histamine (highest dose was 1.0 gamma). There was very marked improvement by the end of May. The last injection (twenty-eighth), 4.0 gamma, was given on July 17, 1939. The child had no allergic symptoms at that time.

CASE 23.—H. W., a 50-year-old woman, has had severe asthma for thirty years. Several attempts at specific desensitization have aggravated her condition considerably. The last

treatment (injections of house dust and feather extracts) had been given eight years prior to her admission to the clinic. The patient's condition had been steadily becoming worse and she was giving herself injections of adrenalin two to three times daily. The physical examination was negative with the exception of wheezing breath sounds. The blood count was normal. Skin tests were negative. Urethane, 2 to 4 Gm. daily, was given symptomatically,²¹ with marked beneficial effect. The first injection of histamine (0.01 gamma) was given on Jan. 9, 1939. Under histamine treatment the patient's condition improved gradually. The attacks became less frequent and less severe. The patient was able to decrease the amount of adrenalin needed to stop the attacks. She had asthmatic attacks through January, but no attacks in February. By the beginning of March she had received 14 injections of histamine, the maximum dose at that time was 0.8 gamma. She had a moderately severe attack on March 3. There was occasional shortness of breath during March, but no real attacks of asthma. The patient from then on improved rapidly. Since she was planning to leave the city in June, the injections were given at twelve-day intervals during April, and at weekly intervals during May. She had no attacks during this time. The last injection (twenty-fifth), 3.0 gamma, was given on June 2. On August 17 she reported slight shortness of breath on two occasions since she was last seen. Her condition was very good and she needed no medication.

COMMENT

Our results with histamine treatment have been very encouraging, especially in a number of cases of asthma of long standing. One of the main problems still confronting us is the question of dosage. We feel that the earlier workers in this field were using too large amounts of histamine and that the dosage suggested by Dzsinich, which we used, is more appropriate.

We believe that some of our therapeutic observations, as for instance in the case of the wheat-sensitive baker, are a further proof of the correctness of the histamine theory of allergic conditions.

SUMMARY

1. The work of Dale and Lewis has laid the foundation for the theory that the symptoms of experimental anaphylaxis and of clinical allergy are due to the liberation of histamine or of a histamine-like substance in sensitive tissues.

2. Various authors have shown that it is possible to induce refractoriness to histamine in human beings and in laboratory animals. Furthermore, it has been demonstrated that it is possible to "desensitize" sensitized guinea pigs nonspecifically by injections of histamine.

3. These observations form the theoretical basis for the treatment of allergic diseases with histamine.

4. The technique of histamine treatment of asthma and vasomotor rhinitis is described, and the results of this treatment are discussed. The results have been very encouraging in a considerable number of instances and warrant further investigation.

REFERENCES

1. Dale, H. H.: Some Chemical Factors in the Control of the Circulation, *Lancet* 1: 1255, 1929.
2. Lewis, T., and Grant, R. T.: Notes on the Anaphylactic Skin Reaction, *Heart* 13: 219, 1926.
3. Hare, R.: An Experimental Investigation Into the Vascular Reactions of the Susceptible Skin to Protein, *Heart* 13: 227, 1926.

4. Duke, W. W.: Urticaria Caused Specifically by Action of Physical Agents, *J. A. M. A.* 83: 349, 1924.
- Bray, G. W.: A Case of Physical Allergy, *J. Allergy* 3: 367, 1932.
- Horton, B. T., Brown, G. E., and Roth, G. M.: Hypersensitiveness to Cold, Etc., *J. A. M. A.* 107: 1263, 1936.
5. Fuehner, H.: Das Pituitrin und seine wirksamen Bestandteile, *München. med. Wehnschr.* 59: 852, 1912.
6. Lewis, T., and Grant, R. T.: Vascular Reactions of the Skin to Injury. II. *Heart* 11: 209, 1924.
7. Farmer, L.: Non-specific "Desensitization" Through Histamine, *J. Immunol.* 36: 37, 1939; 37: 321, 1939.
8. Ramirez, M., and St. George, A. V.: A Contribution to the Etiology of Asthma, *M. J. & Record*, Jan. 16, 1924.
9. Horton, B. T., Mac Lean, A. R., and Craig, W. McK.: A New Syndrome of Vascular Headache, *Proc. Staff Meet., Mayo Clin.* 14: 257, 1939.
10. Friedlaender, W., and Petow, H.: Migräne als Symptom von Cholangio-Hepatopathien und ihre Behandlung mit Histamin, *Med. Klin.* 23: 1498, 1927.
11. Ernestene, A. C., and Banks, B. M.: Use of Histamine in the Treatment of Pruritus, *J. A. M. A.*, p. 328, 1933.
12. Gajdos, A.: "La réactionothérapie de l'urticaire récidivant," *J. d. praticiens* 48: 517, 1934.
13. Joltrain, E.: Histamine et urticaire, *Progrès méd.* p. 164, 1937.
14. Collens, W. S., Lerner, G., and Fialka, S. M.: Insulin Allergy. Treatment With Histamine, *Am. J. M. Sc.* 188: 528, 1934.
15. Stahl, J., and Masson, J.: Histamine et dyspnées asthmatiformes, *Compt. rend. Soc. de biol.* 111: 1003, 1932.
16. Piquet, E.: Asthme et Histamine, Thèse de doctorat, Strasbourg, 1934.
17. Thiberge, N. F.: Histamine and Typhoid Protein Compared in the Control of Asthma and Hay Fever, *J. Allergy* 6: 282, 1935.
18. Dzinich, A.: Die Histaminbehandlung allergischer Zustände, *Klin. Wehnschr.* 14: 1612, 1935.
19. Jacquelin, A.: Asthme et histamine, *Progrès méd.* p. 170, 1937.
20. Openshowski, M.: Histamine—Its Practical Application and the Theory of Its Action, *J. M. Soc. New Jersey* 34: 163, 1937.
21. Farmer, L.: The Use of Urethane in Symptomatic Treatment of Bronchial Asthma, *J. Lab. & Clin. Med.* 24: 453, 1939.

INVESTIGATIONS ON THE RETICULO-ENDOTHELIAL FUNCTION OF CANCER PATIENTS

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THE results of numerous experimental studies,¹ dealing with the role of the reticulo-endothelial system in cancer pathology, suggested to several research workers the possibility of their clinical application. Such investigations might prove of value in two ways: yielding an answer to the questions (a) whether or not a definite and regular involvement of the reticulo-endothelial system is associated with human neoplasia, and (b) if of a positive nature, this evaluation of the reticulo-endothelial function of cancer patients can be utilized for diagnostic and/or prognostic purposes.

Papers on this problem have been published by Krätschell,² Barbera,³ Kavetskiy,⁴ and Schroeder.⁵ All these authors stated on the basis of their work that a definite damage of the reticulo-endothelial function could be found in

*This work was carried out as Research Fellow of the New York City Cancer Hospital and subsequently as Postgraduate Student Observer of the New York University Medical School.

a high percentage of the tested cancer patients. The method generally used in these studies was the Congo red test proposed by Adler and Reimann.⁶ This test is based on the fact that Congo red, a colloidal dye, is stored after intravenous injection by the reticulo-endothelial cells. If, therefore, blood samples are withdrawn shortly after the intravenous injection and later after an interval of time, the concentration of the dye in the blood is found decreased in the second sample, thus permitting an estimate of the per cent of the dye which has been intercepted by the reticulo-endothelial system. The ratio between the dye concentrations in the two samples depends on the storing activity of the reticulo-endothelial system and is termed the Congo red index.

It must be admitted that objections have been raised against the usefulness of the Congo red test for an evaluation of the reticulo-endothelial function.^{7,8} But, on the other hand, animal experiments⁹⁻¹¹ definitely showed the direct connection of the functional activity of the reticulo-endothelial system and the Congo red test. Certainly the colloid-storing ability of this system is only one of its many functions, and it would be of great interest to include these in the clinical examination. But as these immunobiologic, metabolic, hormonal functions of complex character have not as yet been developed as generally applicable functional tests, the present available methods must be utilized. Besides Congo red, other colloids have been proposed for testing the storing capability of the reticulo-endothelial system,¹² but none of these methods appear to be superior to the Congo red test in simplicity and efficiency.

In previous investigations, in collaboration with Willheim,¹³ we applied the Congo red test to rabbits and worked out a modified method which (a) required only small amounts of blood (0.4 c.c. for the single sample, in contrast to the 10 to 20 c.c. of blood necessary in the original method), and (b) permitted a more exact colorimetry by transforming the Congo red into the acid, blue form. Later we published a similar modification¹⁴ which is applicable for clinical use and serves to the same advantage. The usefulness of these modifications has been confirmed by other authors who have used them in animal experiments¹⁵ and for clinical purposes.¹⁶

Because the original studies of the association of reticulo-endothelial function with cancer were carried out on a comparatively small number of cases—in about 50 patients for each experiment—and because we now have at our disposal a more reliable modification of the Congo red test which is also less disturbing to the patient, we were induced to resume the study of this problem and to apply this functional test of the reticulo-endothelial system to a larger group of cancer patients.

Method.—The original test, as well as our modification, is based on the following principle: 10 c.c. of 1 per cent watery Congo red solution are injected intravenously, four and sixty minutes, respectively, after the injection blood samples are withdrawn, and in the serum or plasma the Congo red concentration is colorimetrically determined. As the standard required for the colorimetry must contain the same amount of the patient's serum or plasma, another blood sample is first obtained immediately before the injection of the dye. While in the original method a rather primitive comparative estimation of the red color

of the samples was made, in our modification the Congo red is transformed into the blue compound by addition of hydrochloric acid, before which an acid buffer solution, containing urea, was added, thus (1) diminishing the acid-binding capacity of the serum proteins and (2) preventing their flocculation. Because of the low dye concentration in the samples (2 to 5 milligrams per cent, i.e., 20 to 50 γ in 1 c.c.), it was found necessary for exact colorimetry to add measured amounts of Congo red to the samples. By these procedures clear blue violet hues are obtained which can be easily colorimetrically compared and are proportional to the dye concentration as control experiments proved.

Solutions.—(1) A 1 per cent watery solution can be prepared by exactly weighing Congo red powder and sterilizing in the autoclave. This solution should be used within twenty-four hours and, therefore, must be freshly prepared. For the majority of our experiments we used Loeser's commercial preparation of "1 per cent Congo red."

(2) Sterile 3.8 per cent solution of sodium citrate.

(3) One-tenth per cent and 0.05 per cent Congo red solutions, freshly prepared by dilution of the 1 per cent Congo red solution, used for the injection in the same experiment.

(4) Glycine-hydrochloric acid buffer, containing 25 per cent urea. A mixture of 0.75 Gm. of glycine and 0.59 Gm. of sodium chloride are dissolved in 10 c.c. of water, and 52 parts of this solution are mixed with 48 parts of tenth-normal hydrochloric acid. In 100 c.c. of this mixture 25 Gm. of urea are dissolved. This reagent has a pH of approximately 2, and is usable for several weeks if stored in the icebox.

(5) Ten per cent hydrochloric acid.

Performance of the Test.—To avoid lipemia of the blood samples, the tested patient should be bled either after fasting or at least not shortly after the intake of food.

1. Using a dry 5 c.c. syringe or one rinsed with citrate solution, containing exactly 1 c.c. of 3.8 per cent sodium citrate, blood is aspirated from the cubital vein until the 5 c.c. mark (4 c.c. of blood). Aspiration of air bubbles must be avoided. The contents of the syringe are mixed and put into a dry centrifuging tube marked *Sample 1*.

2. Immediately following, while the needle remains in the vein, 10 c.c. of the 1 per cent Congo red solution are injected.

3. Four minutes after the injection blood is withdrawn in the same way as described above (*Sample 2*).

4. *Sample 3* is obtained sixty minutes after the injection.

Colorimetry.—All three samples are sharply centrifuged. After careful separation from the red blood cells, 2 c.c. of each sample are put into dry test tubes. To the colorless sample 1, serving as standard, 0.1 c.c. of the 0.1 per cent Congo red solution is added with a micropipette; to samples 2 and 3, 0.1 c.c. of the 0.05 per cent Congo red solution, corresponding to 100 and 50 γ , respectively, is added. To each sample is now added 2 c.c. of the buffer-urea solution and 0.5 c.c. of 10 per cent hydrochloric acid. Thus a blue violet color is

developed and the colorimetry is immediately performed, as precipitations may occur after longer delay. Sample 1, containing 100 γ Congo red, is used as standard.

Calculation.—The Congo red content of samples 2 and 3 is found by the equation $x = \frac{1000}{r} \gamma$, if the standard is kept at the mark 10 and r is substituted by the respective reading. From these values, 50 γ , which have been added as previously described, are subtracted for obtaining the originally present Congo red content of the samples. The Congo red index, showing the per cent of decrease of the Congo red and thus serving as measure of the reticulo-endothelial activity, is obtained by dividing the Congo red content of sample 3 by the Congo red content of sample 2 and multiplying this figure by 100.* The Congo red content of the samples, expressed in milligrams per cent, is found by dividing the γ values by 16.

TABLE I
CANCER-FREE PATIENTS

NO.	AGE	DIAGNOSIS	C.R.I.*	NO.	AGE	DIAGNOSIS	C.R.I.*
1	38	Sinusitis	44.9	26	28	Eczema	66.8
2	63	Lipoma of neck	37.6	27	60	Mycosis	63.9
3	39	Ulcer of breast	34.3	28	32	Warts	57.5
4	39	Keloid of ear	33.2	29	37	Menorrhagia	62.9
5	37	Syringomyelia	50.4	30	48	Metrorrhagia	49.6
6	22	Fibroma of nose	56.8	31	21	Acne vulgaris	57.5
7	30	Healthy	45.1	32	71	Dermatitis	65.5
8	48	Eczema	54.6	33	33	Keloid of arm	67.1
9	26	Keloid of ear	57.5	34	57	Warts	55.6
10	60	Neuralgia	62.5	35	56	Endometritis	74.8
11	44	Sebaceous cyst	67.5	36	48	Eczema	62.8
12	73	Morbus frey	62.9	37	24	Keloid of breast	63.1
13	45	Cellulitis of leg	61.3	38	21	Ringworm	52.5
14	39	Sebaceous cyst	63.5	39	28	Eczema	64.1
15	28	Keloid of ear	33.3	40	28	Tuberculosis of rib	60.5
16	45	Eczema	61.3	41	56	Metrorrhagia	75.9
17	39	Boils	32.7	42	59	Fibroid of uterus	52.5
18	37	Psoriasis	62.1	43	58	Dermatitis	55.2
19	43	Herpes zoster	59.8	44	38	Fracture of tibia	56.5
20	64	Dupuytren's contracture	57.1	45	47	Fracture of femur	57.1
21	21	Epidermophytosis	34.8	46	27	Spinal cord lesion	61.9
22	45	Dermatitis	69.7	47	46	Fracture of tibia	51.6
23	67	Mycosis	37.7	48	27	Hallux valgus	62.9
24	44	Mycosis	44.4	49	61	Fracture of femur	50.2
25	20	Acne vulgaris	67.6				

*Congo red index.

Complications.—By strict attention to the above described procedure the plasmas obtained are never hemolytic. Should hemolysis occur, however, this source of error, so important in the original method, is easily detected as the acidification with hydrochloric acid results in a brownish color (hematin), which does not allow a colorimetric comparison. Such a test should be discarded. Strongly icteric or lipemic samples also might prevent a successful colorimetric determination. Finally, it is known that in amyloidosis intravenously injected

*Some authors, ourselves included, in reported animal experiments used a different Congo red index, figuring the per cent of decrease according to the formula: Congo red content of sample 2 minus Congo red content of sample 3 divided by Congo red content of sample 2 and multiplied by 100. This value corresponds to 100 minus the original Congo red index and is the higher, the more Congo red has disappeared.

Congo red disappears very quickly from the blood stream because of a great chemical affinity of Congo red to amyloid (Bennhold's test). In these cases, of course, the Congo red test cannot be used for an evaluation of the reticulo-endothelial function.

Using the described method, we tested 49 patients clinically free of malignancy, and 100 patients suffering from malignant neoplasms. In 12 cancer patients the test was repeatedly performed during and after radiation therapy. The results of these tests may be seen from Tables I to IV.

Table I, comprising the cancer-free patients, exhibits values of the Congo red index ranging from 32.7 to 73.9 per cent. Eight patients (11, 22, 25, 26, 29, 33, 41, 48) show indices near or beyond 70 and correspondingly must be considered as more or less impaired in their reticulo-endothelial function. The normal range varies between 30 and 65 per cent. In our experiments the average value is found as approximately 55 per cent, which means that a little less than half of the injected Congo red amount is stored by the reticulo-endothelial system during the time of the test.

Turning to the neoplastic cases it can be seen that 86 out of the 100 tested patients found in Table II exhibit values ranging between 70 and 100. In 14 cases, however, included in Table III, the Congo red index was found below 70.

On the basis of these results it seems possible to draw the following conclusions:

1. In 86 per cent of the investigated cancer cases a definite severe damage of the reticulo-endothelial function has been found by means of the Congo red test. This damage does not depend on a disturbance of the patient's general condition nor does it seem to be connected with the advancement or the generalization of the malignancy. While at present it cannot be explained why in 14 per cent of our cases this phenomenon could not be observed, the marked involvement of the reticulo-endothelial system in human neoplasia may be considered as an established and probably important factor of cancer pathology. For the time being, however, it cannot be decided if this phenomenon is primarily connected with tumor origin and growth or is only a secondary, concomitant factor. An answer to this question may be looked for from suitable animal experiments.¹⁷

2. A diagnostic value cannot be attributed to the Congo red test both because of its nonspecificity as many other conditions, mainly infections, also result in damaged reticulo-endothelial function, and because in about 14 per cent of our cancer patients the Congo red test failed to reveal an impairment of the reticulo-endothelial system. Therefore, the result of the Congo red test might be evaluated only in combination with other clinical and laboratory findings.

3. On the other hand, a certain prognostic value seems to adhere to the test. By grouping the patients according to their Congo red index and comparing the occurrence of death within the time of observation (six to nine months), we see the following distribution:

Group I:	C.R.I. 70-80	6 out of 20 patients died (30 per cent)
Group II:	C.R.I. 80-90	21 out of 39 patients died (54 per cent)
Group III:	C.R.I. 90-100	19 out of 27 patients died (70 per cent)

TABLE 11

CANCER PATIENTS WITH DAMAGED RETICULO-ENDOTHELIAL FUNCTION

NO.	DATE*	AGE	CANCER OF	C.R.I.	NOTE
1	12/22	45	Stomach	84.7	Died 12/29
2	12/23	78	Rectum	82.4	Unimproved 10/3
3	12/27	38	Rectum	76.1	Died 5/28
4	12/28	68	Prostate	86.2	Unimproved 10/3
5	1/ 3	78	Prostate	87.0	Improved 10/15
6	1/ 5	53	Floor of mouth	91.4	Died 6/17; cf. Table IV
7	1/ 6	68	Nasopharynx	75.2	Discharged unimproved 1/28
8	1/ 9	38	Perineum	88.4	Unimproved 10/3; cf. Table IV
9	1/ 9	64	Tongue	88.0	Died 8/7; cf. Table IV
10	1/10	78	Stomach	100.0	Died 3/19
11	1/11	68	Prostate	88.5	Died 3/6
12	1/12	41	Esophagus	88.3	Died 1/17
13	1/13	38	Breast	95.9	Died 7/4; cf. Table IV
14	1/17	28	Breast	84.6	Died 6/12
15	1/18	53	Rectum	86.7	Improved 9/26
16	1/18	43	Cervix	90.4	Unimproved 10/23
17	1/23	51	Esophagus	90.3	Discharged unimproved 1/31
18	1/24	45	Cervix	92.3	Died 3/29
19	1/25	45	Breast	89.3	Died 3/7
20	1/26	70	Rectum	83.1	Unimproved 12/22
21	1/26	78	Tongue	96.0	Discharged improved 6/24
22	1/27	73	Bladder	81.9	Unimproved 10/4
23	2/ 6	45	Larynx	71.0	Discharged unimproved 2/23
24	2/ 6	68	Tongue	90.5	Died 4/24; cf. Table IV
25	2/ 8	55	Breast	90.7	Died 8/20
26	2/ 9	52	Stomach	94.1	Died 7/2
27	2/ 9	74	Floor of mouth	100.0	Died 10/28; cf. Table IV
28	2/14	60	Stomach	87.5	Died 7/28
29	2/16	72	Tongue	73.7	Improved 5/5
30	2/16	49	Rectum	84.9	Unimproved 2/23
31	2/16	72	Larynx	83.4	Died 11/30
32	2/16	58	Penis	80.1	Died 3/20
33	2/21	46	Larynx	85.9	Died 5/26
34	2/21	58	Larynx	83.4	Unimproved 12/24
35	2/23	70	Rectum	72.6	Discharged unimproved 2/26
36	2/23	62	Lung	83.4	Died 4/13
37	2/23	71	Neck	94.1	Improved 7/6; cf. Table IV
38	2/27	59	Rectum	72.5	Died 10/24
39	2/27	62	Esophagus	94.2	Died 7/23
40	2/28	35	Neck	78.3	Improved 12/26
41	2/28	43	Cervix	95.7	Discharged improved 3/29
42	3/ 1	55	Breast	85.2	Died 5/22
43	3/ 2	69	Prostate	100.0	Died 3/16
44	3/ 2	44	Testis	87.1	Died 3/30
45	3/ 3	46	Larynx	75.7	Discharged improved 5/20
46	3/ 6	76	Breast	84.4	Discharged unimproved 3/11
47	3/ 7	59	Lung	81.8	Died 3/29
48	3/ 9	44	Tonsil	85.2	Died 3/23
49	3/ 9	58	Esophagus	85.3	Died 5/11
50	3/ 9	71	Prostate	90.5	Died 4/9
51	3/10	44	Cervix	80.9	Discharged improved 6/19
52	3/13	42	Breast	72.5	Discharged improved 4/21
53	3/13	59	Cervix	100.0	Died 4/19
54	3/14	69	Stomach	86.7	Discharged unimproved 4/1
55	3/17	70	Stomach	82.6	Discharged unimproved 4/6
56	3/20	66	Larynx	82.2	Discharged improved 4/24
57	3/21	64	Rectum	77.5	Died 4/14
58	3/21	78	Tongue	100.0	Died 4/9
59	3/23	77	Tonsil	85.8	Died 6/24
60	3/27	44	Face	75.4	Unimproved 10/29
61	3/27	84	Larynx	100.0	Died 8/24

TABLE II—CONT'D

NO.	DATE*	AGE	CANCER OF	C.R.I.	NOTE
62	3/28	36	Cervix	78.6	Died 6/24
63	3/30	52	Esophagus	100.0	Died 11/23; cf. Table IV
64	3/31	52	Rectum	92.3	Unimproved 12/23
65	4/ 6	47	Middle ear	90.3	Died 10/29
66	4/ 7	50	Tongue	88.5	Died 7/27; cf. Table IV
67	4/12	51	Tongue	96.9	Discharged improved 7/8
68	4/13	54	Kidney	79.9	Died 8/17
69	4/13	68	Cervix	72.8	Discharged unimproved 7/6
70	4/13	50	Cervix	90.1	Died 10/30
71	4/14	60	Rectum	88.7	Died 4/30
72	4/17	63	Prostate	96.0	Died 5/5
73	4/19	44	Lip	72.7	Improved 7/13
74	4/25	69	Stomach	84.0	Unimproved 10/24
75	4/26	57	Breast	82.3	Unimproved 10/24
76	4/26	62	Lip	71.5	Discharged improved July; cf. Table IV
77	5/ 3	28	Breast	77.1	No follow-up
78	5/ 4	50	Breast	85.4	Unimproved 7/20
79	5/ 8	89	Face	70.5	Unimproved 10/29
80	5/15	46	Breast	86.8	Discharged unimproved 10/1
81	5/25	58	Cheek	81.7	Died 8/19
82	6/21	56	Foot	90.1	Unimproved 12/18
83	6/22	54	Larynx	79.6	Unimproved 12/15
84	6/30	59	Lung	100.0	Died 7/18
85	6/30	61	Prostate	78.0	Unimproved 12/7
86	6/30	35	Lung	85.2	Died 7/13

TABLE III

"NEGATIVE" CANCER PATIENTS

NO.	DATE*	AGE	CANCER OF	C.R.I.	NOTE
1	1/12	56	Sigmoid	68.9	Improved 12/26
2	1/16	38	Cervix	66.8	Died 2/15
3	1/17	23	Cervix	56.5	Discharged improved 2/15
4	1/31	57	Larynx	58.6	Died 6/2
5	2/ 6	38	Lung	46.2	Discharged improved 6/23
6	2/ 7	50	Ovary	64.4	Discharged unimproved 2/15
7	2/15	48	Cervix	61.9	Died 4/29
8	3/ 1	53	Breast	46.3	Discharged unimproved 6/6
9	3/ 3	65	Cheek	67.2	Died 4/23
10	3/15	67	Colon	68.8	Died 6/22
11	3/15	59	Rectum	52.3	Died 3/18
12	3/16	38	Breast	66.6	Died 6/13
13	4/24	51	Tongue	60.0	Improved 12/13
14	5/10	49	Lip	65.3	Improved 12/19

*All dates refer to 1939 with exception of patients 1 to 4.

Therefore, the more severe disturbance of the reticulo-endothelial system appears to be connected with a more rapid fatal progress of the disease.

4. As shown in Table IV, the Congo red test was also repeatedly carried out in 12 cancer patients before, during, and after their treatment with radiation therapy. Here we see that in 2 patients (21, 37) the clinical improvement, achieved by the therapy, was also accompanied by a reversion of the Congo red index to normal values. In 2 patients (27, 66) the Congo red index was lowered, coinciding with a clinically observed radiation reaction and indicating an improved function of the reticulo-endothelial system, but no lasting clinical improvement was brought about. In the rest of the patients no clinical improvement was observed and the repeated performance of the Congo red test always

exhibited a damage of the reticulo-endothelial function. In patient 13 the regression of a breast lesion was also expressed by an improved reticulo-endothelial function, but the finally lethal recurrence was foreshadowed by an increase of the Congo red index, characteristic for the failure of the reticulo-endothelial function. As far as these few investigations permit us to judge, the repeated performance of the Congo red test during a treatment would prove valuable for prognostic purposes. In this connection it may be mentioned that these results support the opinion that successful radation therapy is accompanied by an activation of the reticulo-endothelial system or is partly dependent on it (Caspari,¹⁸ Lewin¹⁹).

TABLE IV
TESTS REPEATED DURING TREATMENT OF CANCER PATIENTS

NO.*	DATE	CANCER OF	C.R.I.	TREATMENT	NOTE
6	1/ 5 4/17	Floor of mouth	91.4 90.6	X-ray: 1/8-2/28	Died 6/17
8	1/ 9 3/30 6/12	Perineum	88.4 83.1 94.2	X-ray: 4/30-6/28	Biopsy 5/9, malignancy present; identical finding 8/5
9	1/ 9 3/23	Tongue	88.0 87.8	X-ray: 2/17-3/28	Died 8/7
13	1/13 3/ 6 6/13	Breast	95.9 72.3 88.6	X-ray: 12/16, 38-6/28	Local condition healed 2/28; recurrence 6/30; died 7/4
21	1/26 3/ 6 6/12	Tongue	96.0 88.8 65.6	X-ray: 1/26-3/20; 4/10-4/20	Discharged improved 6/24
24	2/ 6 3/ 8	Tongue	90.5 86.3	X-ray: 2/23-3/29	Died 4/24
27	2/ 9 3/22 6/ 8	Floor of mouth	100.0 54.3 62.6	X-ray 2/15-3/15; radium mold: 5/15-5/19	Height of radiation reaction 3/22; marked regression 6/26; died 10/28
37	2/23 3/27 6/ 8	Neck	94.1 80.6 40.9	X-ray: 2/27-3/21; 5/24-6/25	Discharged highly improved 7/6
63	3/30 6/ 6	Esophagus	100.0 88.5	X-ray: 4/10-8/23	Died 11/23
66	4/ 7 6/ 8	Tongue	88.5 61.6	X-ray: 4/10-6/15	Died 7/27
76	4/26 6/14	Lip	71.5 74.8	Radium: May '39	Discharged improved July '39
78	5/ 4 6/14	Breast	85.4 71.5	X-ray: 5/10-6/15	No follow-up

*Numbers refer to corresponding cases in Table II.

5. In order to include a complete enumeration of our studies, we refer to the less conclusive results obtained by applying the Congo red test to 5 patients with sarcoma. Only in 2 of these was a decreased reticulo-endothelial function found: reticulum cell sarcoma, C.R.I. 85.8; fibromyxosarcoma, C.R.I. 100. In a case of fibrosarcoma of the intestines complete disappearance of the dye in the blood was observed after sixty minutes in a Congo red test performed twice (C.R.I. 0). No amyloidosis was found in the autopsy of this patient. In a patient with round-cell sarcoma a C.R.I. of 38.8, and in one with a reticulum-cell sarcoma a C.R.I. of 48.9 were found. While the number of the sarcoma patients studied is far too small to permit us drawing any conclusion, it does not seem

altogether improbable that the reticuloendothelial function is less frequently involved in sarcomas of mesodermal origin than in epithelial neoplasia. A similar view was expressed by Cramer²⁰ concerning the interpretation of reticuloendothelial studies made on rat sarcomas.

SUMMARY

A modification of the Congo red test has been described which can be used for clinical testing of the reticuloendothelial function. This test has been applied to 49 persons free of malignancy and to 100 patients afflicted with malignant neoplasms. A damaged function of the reticuloendothelial system was found in 86 per cent of the cancer cases. The possibility of a prognostic evaluation of this test was discussed as to duration of life and effectiveness of radiation therapy.

REFERENCES

1. Willheim, R., and Stern, K.: Wege und Ergebnisse chemischer Krebsforschung, Vienna, 1936, Aeskulap-Verlag, pp. 355-378.
2. Krätschell, U.: Ueber das Verhalten des retikulo-endothelialen Systems bei malignen Tumoren an Hand der Kongorot Probe, Fortschr. d. Med. 51: 983, 1933.
3. Barbera, S.: Esplorazione funzionale del sistema reticulo-endoteliale col rosso congo, Gazz. internaz. med.-chir. 45: 710, 1935.
4. Kavetskiy, R. E.: Functional State of Connective Tissue During Cancerization Process, Med. zhur. 7: 91, 1937.
5. Schroeder, W.: Ueber den Wert der Kongorot Probe bei Krebskranken, Klin. Wchnschr. 18: 248, 1939.
6. Adler, H., and Reimann, F.: Beitrag zur Funktionspruefung des reticulo-endothelialen Apparates, Ztschr. f. d. ges. exper. Med. 47: 617, 1925.
7. Letterer, E.: Die Funktionspruefung mit Kongorot, Ztschr. f. d. ges. exper. Med. 93: 103, 1934.
8. Ajello, L.: Sul valore della prova col rosso congo, Diag. e tec. di lab. 6: 732, 1935.
9. Moncorps, C., and Guenther, O.: Chemische Identifikation einer aus der Milz isolierten, die mesenchymale Abfangfunktion steigernden Substanz als 2-oxy-6-aminopyrimidin (cytosin), Klin. Wchnschr. 12: 979, 1933.
10. Goebel, F., and Miller, J. M.: L'ablation de la rate et le système réticulo-endothéliale, Compt. rend. Soc. de biol. 119: 775, 1935.
11. del Zoppo, R.: La prova del rosso congo, Folia med. 21: 33, 1935; Policlinico (sez. prat.) 42: 89, 1935.
12. Brumann, H.: Methoden zur Untersuchung des R. E. S., Abderhalden's Arbeitsmethoden, Abtl. 8, Tl. 1, H. 7, Lief. 383.
13. Stern, K., and Willheim, R.: Beeinflussung des reticulo-endothelialen Systems durch Carotin, Ztschr. f. d. exper. Med. 97: 354, 1935.
14. Stern, K.: Zur Methodik der klinischen Funktionspruefung des reticulo-endothelialen Systems mittels Kongorot, Wien. klin. Wchnschr. 50: 1579, 1937.
15. Wetzler-Ligeti, C., and Wiesner, B. P.: Restropic Effects of Anterior Lobe Extracts, Endocrinology 22: 693, 1938.
16. Csato, T., Wetzler-Ligeti, C., and Wiesner, B. P.: Effect of the Prolonged Administration of 1:2:5:6-Dibenzanthracene on the Reticulo-Endothelial System of Rabbits, Am. J. Cancer 36: 257, 1939.
17. Haas, F.: Unpublished study.
18. Stern, K.: Aspects on the Relationship Between Reticulo-Endothelial System and Malignancy, New Internat. Clin. In press.
19. Capari, W.: Weiteres zur biologischen Grundlage der Strahlenwirkung, Strahlentherapie 18: 17, 1924.
20. Lenja, C.: Die Immunitaetsvorgaenge bei malignen Tumoren, Ztschr. f. Krebsforsch. 27: 133, 1928.
21. Cramer, H.: Unspezifische Krebstherapie, München. med. Wchnschr. 84: 943, 1937.

CLINICAL CHEMISTRY

VITAMIN K ACTIVITY OF 2-METHYL-1, 4-NAPHTHOQUINONE AND 4-AMINO-2-METHYL-1-NAPHTHOL IN HYPOPROTHROMBINEMIA*

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IN VIEW of the promising preventive and curative role of vitamin K in hypoprothrombinemia, a brief communication is submitted on the recent chemical developments and the clinical status of synthetic vitamin K and substitute compounds.

MODE OF STUDY

Twenty-seven patients having hypoprothrombinemia below 40 per cent are reported. Specimens of blood were obtained by venipuncture during the morning hours. The prothrombin determinations were made by the method of Quick¹ and repeated at twenty-four-hour intervals when feasible. The normal clotting time for the method throughout the study was thirteen seconds, representing 100 per cent. Since many clinical variables must be considered in their eventual evaluation, this study involved only a preliminary assessment of activity of the chemical substances described when used in states of hypoprothrombinemia below 40 per cent of normal.

Dam² and Stokstad³ are identified with the early phases of research on a factor having antihemorrhagic properties. The former demonstrated its presence in vegetables and alfalfa. Almquist and Stokstad⁴ showed that a small percentage of alfalfa in the deficient diet prevented hemorrhagic tendency in chicks. Various contributions on the chemical nature of vitamin K cited by Doisy and his associates in a recent review⁵ preceded the investigations of McKee, Binkley, Thayer, MacCorquodale, and Doisy,⁶ and Binkley, MacCorquodale, Thayer, and Doisy⁷ which led to the isolation of vitamins K₁ and K₂.

Almquist and Klose⁸ reported on the antihemorrhagic property of a synthetic naphthoquinone, "phthiocol," 2-methyl-3-hydroxy-1, 4-naphthoquinone, which was isolated from the tubercle bacillus by Anderson and Newman⁹ in 1933. These former workers considered "phthiocol" to be the simplest member of a homologous series of antihemorrhagic substances. They found it possessed the same degree of potency whether given orally, intramuscularly, or intravenously, but it had only 1/100 to 1/20 the activity of vitamin K derived from alfalfa.

In May, 1939, Doisy and his co-workers^{11, 12} announced the isolation of two forms of vitamin K: K₁ from alfalfa and K₂ from fermented fish meal. As

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result of chemical analysis it was suggested that K_1 and K_2 possessed quinonoid structures. Further study demonstrated vitamin K to be disubstituted 1, 4-naphthoquinone.

Doisy and his co-workers assigned to vitamin K derived from alfalfa the chemical formula of 2-methyl-3-phytyl-1, 4-naphthoquinone. This substance has been synthesized and when used in this pure form orally has been found to induce remission of hypoprothrombinemia in animals and man. It is oil soluble and stable in oily solution, assaying 1,000 curative chick units* per milligram. While not fully evaluated clinically, a dose range of 6,000 to 9,000 units daily is effective when the prothrombin deficiency is below 40 per cent of normal.

Further research on substances having quinonoid structures by Ansbaeher and Fernholz¹³ demonstrated that 2-methyl-1, 4-naphthoquinone, first discovered by Fries and Lohmann in 1921,¹⁴ possessed vitamin K activity. Each milligram of this substance assays about 2,000 curative chick units of vitamin K as determined by the seventy-two-hour method. It exists as bright yellow, prismatic crystals, possesses a quinone-like odor, and is affected by light; for example, exposure of the material in a glass-stoppered bottle to diffuse light for about two weeks not only changes the color to tan or off-white, but also raises the melting point from the normal range, 105° to 107° C., up to 170° C.

TABLE I

INEFFECTIVENESS OF 2-METHYL-1, 4-NAPHTHOQUINONE ORALLY IN DRY FORM IN HYPOPROTHROMBINEMIA OF OBSTRUCTIVE JAUNDICE AND BILIARY CIRRHOSIS

PATIENT NO.	PROTHROMBIN %		DAYS TREATED	DAILY DOSAGE (C.C.U.)*
	BEFORE	AFTER		
101	18	50	6	1,200
2Yo	18	25	11	2,400
32a	40	50	7	2,400
32a	40	50	9	7,200
4St	30	35	5	2,400
4St	30	35	7	3,600
5Ba	30	20	3	7,200
6Met	40	20	14	2,400
7Pot	15	22	3	3,600
	25	30	14	2,400

*Curative chick units.

†Biliary cirrhosis.

The compound 2-methyl-1, 4-naphthoquinone is soluble in oil and manifests antihemorrhagic action when given orally. Sharp¹⁵ reported that doses of 1,200 to 7,200 curative chick units daily, when given orally in dry form, with or without whole bile, caused little alteration in hypoprothrombinemia associated with obstructive jaundice and biliary cirrhosis (Table I). When the same patients, however, received 2-methyl-1, 4-naphthoquinone dissolved in corn oil, a daily dosage of 2,400 to 4,800 units restored prothrombin to a satisfactory level within three to five days (Table II). Rhoads and Flicgelman¹⁶ have reported favorably on the use of 2-methyl-1, 4-naphthoquinone in prothrombin deficiency. In daily doses of 1 to 4 mg. orally only one of ten patients failed to respond and no toxic effects were encountered.

*The curative chick unit of vitamin K (J. Biol. Chem. 123: CXXI, 1938) may be described as the smallest quantity of a vitamin K preparation given over a three-day period which will produce an average coagulation time of ten minutes or less in at least 50 per cent of baby chicks which have been fed a vitamin K-free diet for the first fourteen to fifteen days. The unit corresponds to the activity of approximately 50 mg. of dry alfalfa.

TABLE II

EFFECTIVENESS OF 2-METHYL-1, 4-NAPHTHOQUINONE IN HYPOPROTHROMBINEMIA OF OBSTRUCTIVE JAUNDICE WHEN DISSOLVED IN OIL AND GIVEN ORALLY
(Cases 101, 2Yo, 3Za, 4St, and 5Ba from Table 1)

PATIENT NO.	PROTHROMBIN %		DAYS TREATED	DAILY DOSAGE (C.C.)
	BEFORE	AFTER		
101	30	80	3	2,400
3Za	38	90	6	2,400
4St	18	70	8	3,600
6Ti	28	90	3	3,600
7Bo	25	90	6	4,800
8Du	32	70	6	4,800
9Re	30	80	3	4,800
10Ha	28	70	10	4,800
11We	30	70	3	4,800
2Yo	35	65	7	7,200
5Ba	25	75	7	9,600

TABLE III

EFFECT OF 4-AMINO-2-METHYL-1-NAPHTHOL IN HYPOPROTHROMBINEMIA WHEN GIVEN INTRAVENOUSLY

PATIENT NO.	AGE	SEX	DIAGNOSIS	PROTHROMBIN %		DAYS TREATMENT	TOTAL DOSAGE VIT. K (MG.)	REMARKS
				BEFORE	AFTER			
1Mac	59 yr.	♀	Atrophic liver—C*	15	15	7	28	Died
2El	12 days	♂	Hemorrhagic disease of newborn	27	100	1	1	
3Tu	73 yr.	♂	Obstructive jaundice —C	40	70	11	22	Recovered
3Tu	73 yr.	♂	Second day post-operative	—	40	—	None	
3Tu	73 yr.	♂	Postoperative	40	80	14	28	
4Ga	25 yr.	♂	Catarrhal jaundice	50	90	4	8	
5Ev	39 yr.	♀	Cholecystitis with stone—T†	50	50	8	16	
6Kc	30 yr.	♂	Ulcerative colitis—C	50	50	11	44	No effect of vitamin K orally
7Wa	57 yr.	♀	Cholangitis, chronic —T	28	60	3	6	
8St	62 yr.	♂	Hernia, strangulated —C	10	45	2	6	Died
9Ye	43 yr.	♂	Hernia, strangulated —C	18	45	6	12	
10St	51 yr.	♂	Obstructive jaundice —C	50	60	4	16	
11Ma	56 yr.	♀	Obstructive jaundice —C	5	80	6	12	
12Bu	3 days	♂	Hemorrhagic disease of newborn	No clot	80	1	6	
13Al	3 days	♀	Hemorrhagic disease of newborn	10	90	1	2	
14McF	31 yr.	♂	Gunshot wound liver —C	30	80	2	4	

*Confirmed.

†Tentative.

Given in doses of several milligrams daily for three to four weeks to numerous adult patients, 2-methyl-1, 4-naphthoquinone has developed no manifestations of intolerance. Rhoads and Fliegelman in their communication stated that they had given 0.5 mg. per kilogram of body weight without producing any

untoward phenomenon. It is the most potent of the oil-soluble vitamin K substances as judged by limited clinical experience.

Parenteral 4-Amino-2-Methyl-1-Naphthol Hydrochloride.—Further investigation of chemical substances having antihemorrhagic properties of vitamin K was continued in an effort to find a water-soluble and stable compound for parenteral use. This research resulted in the synthesis of 4-amino-2-methyl-1-naphthol and 4-amino-3-methyl-1-naphthol¹⁷ as the hydrochloride, both of which are water soluble and stable. It was found, however, that the former possessed definitely greater vitamin K activity by the curative chick method than the latter—actually almost three times greater than K₁.¹⁸ In Table III are given data on the use of 4-amino-2-methyl-1-naphthol hydrochloride in hypoprothrombinemia in man. Daily doses ranging from 1 mg. to 6 mg. (1,800 to 10,800 curative chick units in the form of the hydrochloride) given intravenously restored to normal severe prothrombin deficiency of obstructive jaundice within two to three days, while hemorrhagic states of the newborn responded within twelve hours to a dosage of 1 mg. intravenously (1,800 units).

DISCUSSION

Warner, Brinkhous, and Smith¹⁹ have reported extensive observations on the antihemorrhage effect of vitamin K in obstructive jaundice, as have Butt, Snell, and Osterberg.²⁰ Hellman and Shettles,²¹ and Waddell and Guerry²² have studied hypoprothrombinemia in expectant mothers and in the newborn. Hellman and Shettles, using vitamin K concentrate, demonstrated that the plasma prothrombin of the newborn can be raised by administration to the mother prior to delivery and by direct use in the infant. Waddell and Guerry also demonstrated therapeutic effectiveness of vitamin K concentrate and 2-methyl-1, 4-naphthoquinone on prothrombin deficiency in the newborn, and that prenatal administration may prevent intracranial hemorrhage associated with normal delivery.

Our unpublished observations on several hundred adult patients show that depleted prothrombin is not uncommon in blood disorders associated with or without jaundice. Hypoprothrombinemia in some of these conditions has responded to vitamin K, but a complete evaluation must be deferred.

The isolation and synthesis of vitamins K₁ and K₂ constitute an epochal chemical achievement. Projection of chemical research beyond this phase, however, promises therapeutic affluence within the partially defined limits of usefulness of antihemorrhagic factors.

The effectiveness of parenteral 4-amino-2-methyl-1-naphthol, the absence of signs of intolerance, and its rapidity of action imply that prevention of hypoprothrombinic bleeding in the newborn and adult will be a practical procedure. Further, that the control of bleeding associated with jaundice when surgical measures are urgent can be expected by the use of potent synthetic vitamin K substances by either the oral or the parenteral route of administration.

SUMMARY

A brief description of the chemical status of vitamin K₁ and synthetic substitutes having similar antihemorrhagic activity is given.

Seven patients having hypoprothrombinemia were ineffectively treated orally with 2-methyl-1, 4-naphthoquinone in dry form.

Five of the first series of seven patients and an additional six patients having prothrombin deficiency were successfully treated orally with 2-methyl-1, 4-naphthoquinone when the substance was dissolved in oil. No toxic effects were detected.

A water-soluble substance, 4-amino-2-methyl-1-naphthol hydrochloride, when given intravenously in hypoprothrombinemia, was effective in a majority of fourteen patients. Hemorrhagic tendency of the newborn responded rapidly. It has not produced toxic manifestations in the dosage used.

REFERENCES

1. Quick, A. J.: The Nature of the Bleeding in Jaundice, *J. A. M. A.* 110: 1658, 1938.
2. Dam, H.: (a) Cholesterinstoffwechsel in Hühnereiern und Hühnchen, *Biochem. Ztschr.* 215: 475, 1929; (b) Über die Cholesterinsynthese in Tierkörper, *Biochem. Ztschr.* 220: 158, 1930.
3. Almquist, H. J., and Stokstad, E. L. R.: Dietary Hemorrhagic Disease in Chicks, *Nature* 136: 31, 1935.
4. Almquist, H. J., and Stokstad, E. L. R.: Hemorrhagic Chick Disease of Dietary Origin, *J. Biol. Chem.* 111: 105, 1935.
5. Doisy, E. A., Binkley, S. B., Thayer, S. A., and McKee, R. W.: Vitamin K, *Science* 91: 58, 1940.
6. McKee, R. W., Binkley, S. B., Thayer, S. A., MacCorquodale, D. W., and Doisy, E. A.: The Isolation of Vitamin K₂, *J. Biol. Chem.* 131: 327, 1939.
7. Binkley, S. B., MacCorquodale, D. W., Thayer, S. A., and Doisy, E. A.: The Isolation of Vitamin K₁, *J. Biol. Chem.* 130: 219, 1939.
8. Almquist, H. J., and Klose, A. A.: Color Reactions in Vitamin K Concentrates, *J. Am. Chem. Soc.* 61: 1611, 1939.
9. Anderson, R. J., and Newman, M. S.: Isolation of a Pigment and of Anisic Acid From the Acetone-Soluble Fat of the Human Tubercle Bacillus, *J. Biol. Chem.* 101: 773, 1933.
10. Almquist, H. J., and Klose, A. A.: The Antihemorrhagic Activity of Certain Naphthoquinones, *J. Am. Chem. Soc.* 61: 1923, 1939.
11. McKee, R. W., Binkley, S. B., MacCorquodale, D. W., Thayer, S. A., and Doisy, E. A.: The Isolation of Vitamins K₁ and K₂, *J. Am. Chem. Soc.* 61: 1295, 1939.
12. MacCorquodale, D. W., Binkley, S. B., Thayer, S. A., and Doisy, E. A.: On the Constitution of Vitamin K₁, *J. Am. Chem. Soc.* 61: 1928, 1939.
13. Ansbacher, S., and Fernholz, E.: Simple Compounds With Vitamin K Activity, *J. Am. Chem. Soc.* 61: 1924, 1939.
14. Fries, K., and Lohmann, W.: Untersuchungen in der Reihe des 2-Methyl-Naphthaline, *Ber. Chem. Ges.* 54: 2912, 1921.
15. Sharp, E. A.: Vitamin K Activity of 2-Methyl-1, 4-Naphthoquinone, *Proc. Cen. Soc. Clin. Res. J. A. M. A.* 114: 439, 1940.
16. Rhoads, J. E., and Fliegelman, M. T.: The Use of 2-Methyl-1, 4-Naphthoquinone (A Synthetic Vitamin K Substitute) in the Treatment of Prothrombin Deficiency in Patients, *J. A. M. A.* 114: 400, 1940.
17. Doisy, E. A., MacCorquodale, D. W., Thayer, S. A., Binkley, S. B., and McKee, R. W.: The Isolation, Constitution and Synthesis of Vitamin K₁, *Science* 90: 407, 1939.
18. Emmett, A. D., Brown, R. A., and Kamm, O.: Comparison of the Antihemorrhagic Activity of Natural and Synthetic Vitamin K₁ With the Proposed Standard 2-Methyl-1, 4-Naphthoquinone, *J. Biol. Chem.* 132: 467, 1940.
19. Warner, E. D., Brinkhous, K. M., and Smith, H. P.: Bleeding Tendency of Obstructive Jaundice: Prothrombin Deficiency and Dietary Factors, *Proc. Soc. Exper. Biol. & Med.* 37: 628, 1938.
20. Butt, H. R., Snell, A. M., and Osterberg, A. E.: (a) The Use of Vitamin K and Bile in Treatment of the Hemorrhagic Diathesis in Cases of Jaundice, *Proc. Staff Meet. Mayo Clin.* 13: 74, 1938; (b) Snell, A. M., Butt, H. R., and Osterberg, A. E.: Treatment of the Hemorrhagic Tendency in Jaundice, With Special Reference to Vitamin K, *Am. J. Digest. Dis. & Nutrition* 5: 590, 1938.
21. Hellman, L. M., and Shettles, L. B.: Factors Influencing Plasma Prothrombin in the Newborn Infant, *Bull. Johns Hopkins Hosp.* 65: 138, 1939.
22. Waddell, W. W., Jr., and Guerry, D., III: The Role of Vitamin K in the Etiology, Prevention and Treatment of Hemorrhage in the Newborn Infant, *J. Pediat.* 15: 802, 1939.

MEAT EXTRACTIVES IN STUDIES OF GASTRIC FUNCTION*

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IT HAS long been recognized that meat extractives increase gastric secretion, but until recently there have appeared few data on the quantitative measurement of this response. Tessieri (1930)⁶ stated that ingestion of peptone preparations increased the duration and quantity of human gastric secretion. Komm (1931)⁴ reported that "Tago," a commercial meat hydrolysate, caused a marked increase in the gastric secretion of dogs. Smith and Cowgill (1933)⁵ reported the results of preliminary studies on gastric secretion following administration of various meat extracts. Wilhelmj, O'Brien, and Hill (1936)⁸ advocated a test meal of meat extractives and described its effect on gastric secretion in dogs.

The most important work of a quantitative nature which has been done on human beings appeared in 1939, when Upham and Spindler,⁷ using the test meal as evolved by Wilhelmj and associates, reported their studies of the response of the normal and of certain pathologic stomachs to the meat extractive secretagogue meal. This test meal was prepared by a rather lengthy and laborious treatment of a commercially available meat paste.

Meat extracts have been marketed for over sixty years and are prescribed in certain types of gastrointestinal dysfunction. Little is known, however, of the mechanism involved in the beneficial effects resulting from this therapy. This study was undertaken, therefore, with the dual purpose of obtaining quantitative data on the secretagogue power of a commercially available meat extract which would require little or no chemical treatment before administration as a test meal and of gaining information concerning the gastric response to the administration of a meat extract with the hope of explaining to some extent the mechanism of its action.

PROCEDURE

The test subjects used were healthy male medical students, acquainted with the use of the Rehffuss tube and the purposes of the investigation. The nature of the meals used, the lack of discomfort during the test period, and the reversal of the order of administration of test meal and control meal in one-half of the persons minimized and neutralized any possible psychic disturbances of gastric secretion. In fact, in individuals who were used repeatedly for the same test meal, the striking similarity of the secretory responses (noted by other observers) showed that our results were valid for comparative purposes.

The technique followed the usual procedure, viz., the introduction of the Rehffuss tube, aspiration of fasting contents after a rest of fifteen minutes, and

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the introduction of the previously warmed fluid meal via the tube. Samples were removed at fifteen minutes, thirty minutes, and at thirty-minute intervals thereafter. Care was taken to mix the stomach contents well before withdrawing each sample.

The meals used were: (a) The control meal, prepared by adding 25 Gm. of Ralston (a wheat cereal) to 750 c.c. of boiling water, boiling with stirring for ten minutes, allowing to stand for several hours and decanting 400 c.c. of supernatant fluid. To this was added 1 c.c. of phenol red (6 mg. per cubic centimeter, Hynson, Westcott, and Dunning). This preparation was cloudy owing to suspended starchy material. (b) The meat extract meal (used for series I) was prepared by the addition of 16 c.c. of meat extract,* neutralized to Töpfer's reagent with 5 N hydrochloric acid, to sufficient gruel (prepared as above) to make a total volume of 400 c.c.

Free acidity of the samples withdrawn from the stomach was determined by dilution and titration with fresh 0.01 N sodium hydroxide solution, using Töpfer's reagent as indicator; total chlorides were determined by the Van Slyke and Sendroy modification of Volhard's method; pepsin was determined by Mett's method. The amount of dilution of the gastric contents by various secretions was determined in the samples by comparison in a microcolorimeter of the color of the phenol red in the test meal (after alkalinization) with that of the standard meal used (also after alkalinization to bring out the color).

In series II and III where meat extract, diluted with water instead of gruel meal, was used, it was possible to determine the dilution with fair accuracy without any treatment other than the addition of alkali. Sometimes the brownish color of the standard was matched by adding minute amounts of meat juice to the unknown. Samples containing bile were matched by adding a trace of picramic acid to the standard. Where the suspended starchy material in the gruel meals interfered with color comparison, it was removed before alkalinization by suction filtration through asbestos mats. In most cases, however, an accuracy of 2.5 per cent could be obtained without such treatment, and it was felt that in laboratory practice the loss of time required to attain further accuracy would not be justified.

RESULTS

Fig. 1 shows the curves obtained by averaging the values for all samples at corresponding intervals in ten pairs of test meals. In plotting the total chloride curve for the meat extract meal, the analytical values obtained on the samples were first corrected for the chlorides contained in the test meal before ingestion (due to salt in the meat extract and to chlorides added as hydrochloric acid in neutralization to Töpfer's reagent). The following formula was used:

$$Cl_{int.} = A - A_0 X.$$

A represents the chloride concentration of the sample analyzed; X, the per cent of indicator present in the sample analyzed; and A_0 , the chloride concentration of the original test meal. (A_0 for the meal used in this investigation was 47 c.c. of 0.1 normal chloride per 100 c.c. of test meal.) Actually in in-

*The meat extract used in this investigation was kindly donated by the Valentine's Meat Juice Co., Richmond, Va.

dividual test meals somewhat higher peak values were often observed, but their distribution in the samples of different time intervals resulted in the average values shown.

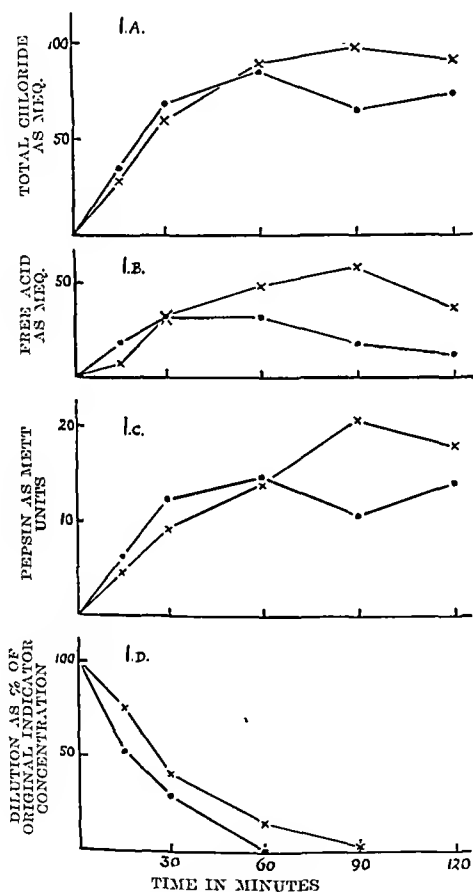


Fig. 1.—x x Gruel plus meat juice extract. • • Gruel meal.

As Fig. 1 shows, in the meat extract meal, and especially in the second hour, there is a higher concentration of total chloride, free acid, and pepsin. These differences occur only after the stomachs have emptied. This state of affairs might have been brought about by one of several things:

1. A greater secretion of acid gastric juice into the *meat extract* meal. This would also lead to a greater dilution of the meat extract meal than of the gruel meal, whereas actually the reverse has occurred (Fig. 1 D).

2. A greater secretion into the *gruel meal* of a chloride solution containing less hydrochloric acid than that secreted in the first hour. Since, however, in gastric juice of varying hydrochloric acid content the hydrochloric acid plus sodium chloride equals a constant of about 0.170 normal (Hollander),² the total chloride of the gastric secretion must remain unchanged. On the contrary Fig 1 A shows that in the case of the gruel meal the total chloride diminished rapidly in the second hour.

3. A regurgitation of duodenal fluids containing chloride and bicarbonate into the gruel meal. This possibility is the only one that can account for all the phenomena found, i.e., the lower total chloride, the relatively still lower free acid, the lower pepsin, and the greater dilution of stomach contents.

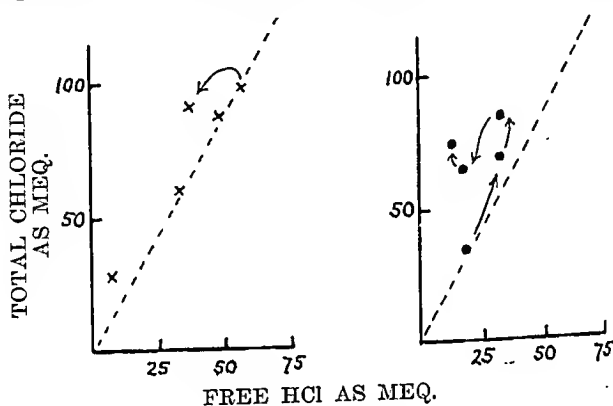


Fig. 2.—x x Gruel plus meat juice extract. • • Gruel meal.

In Fig. 2 these results, at least as regards the relationship of total chloride to free acid, have been compared in another way, by plotting the latter against the former. Hollander (1932)^{2,3} has shown that when various samples of pure gastric juice are drawn from animals, the plot of total chlorides against the corresponding free acid yields a straight line relationship. In our gruel and meat extract meal, where the gastric chloride and acid are diluted only by the fluid meal during the first hour, a similar straight line relationship exists; in the second hour other factors begin to operate, with fall of acidity. In the gruel meal, however, the free acidity very soon falls to the left of the straight line showing that alkalis (presumably of duodenal origin) are neutralizing the free acid only half an hour after the meal was placed in the stomach.

Apperly and Semmens (1928)¹ showed that high total chlorides, high acidity, and increased emptying time in healthy stomachs, indicate a high gastric muscle tonus. They further showed that the duodenum is not so readily able to regurgitate its contents into a hypertonic, relatively contracted stomach. This results in a higher acidity of the stomach contents, which in turn, as is well known, delays the emptying time, as judged by the disappearance of food starch, or dye from the stomach. These are the conditions found in the meat extract meal, which would, therefore, appear to be directly or indirectly responsible for the increased tonicity of the stomach. Clinically it is well known that the latter manifests itself in increased appetite and sense of well-being and thus probably accounts for the popularity of meat extracts during etc.

valescence and poor health. The increased concentration of acid and pepsin and the slower emptying time would also increase the rate and degree of digestion of certain foods.

A further series of test meals (series II) was prepared by dilution of 16 c.c. of meat extract to 399 c.c. with distilled water, and the addition of 1 c.c. of the phenol red solution as in the other test meals, but the results were not significantly different from those in series I. The peak acidity and pepsin values were reached sooner, but not as soon as with the gruel meal alone. Their values were approximately the same. Emptying time also was less than with the combination meal, but showed a delay when compared with the gruel meal.

A third (series III), using a 1:50 dilution of the meat juice, was done in order to study the effect of greater dilution of the meat extractives, the results being similar to those in series II, except that the peak values for concentration of secreted substances were reduced, indicating that the maximum effect of the meat extract was not being obtained. Since use of diluted meat extract alone produces results similar to those found with gruel plus meat extract, the use of meat extract alone as a test meal is advocated. Further work on this test meal is contemplated.

SUMMARY

An examination of the mode of action of a commercially obtainable meat extract was made in order to ascertain the physical basis, if any, for the commonly alleged sense of well-being accompanying the use of such extracts.

In comparison with the ordinary test meal alone, the addition of meat extract to the meal raises the concentration of total chlorides, free acid, and pepsin, and retards the dilution of the gastric contents. These facts are interpreted as indicating an increased tonicity of gastric muscle, which is known to be the physical basis of appetite.

Our experience with meat extract leads us to prefer this substance to all others for routine and experimental test meal work.

We wish to express our appreciation to Dr J. C. Forbes for his helpful suggestions during the course of this work.

REFERENCES

1. Apperly, F. L., and Semmens, K. M.: Variations in Normal Gastric Functions and Their Causes. Some New Experimental and Clinical Observations, M. J. Australia 2: 226, 1928.
2. Hollander, F.: Studies in Gastric Secretion. IV. Variations in the Chlorine Content of Gastric Juice and Their Significance, J. Biol. Chem. 97: 585, 1932.
3. Hollander, F., and Cowgill, G. R.: Studies in Gastric Secretion. I. Gastric Juice of Constant Acidity, J. Biol. Chem. 91: 151, 1931.
4. Kohn, E.: Über die Einwirkung von Fleischhydrolysaten auf die Magensaftabsonderung, Klin. Wchnschr. 10: 981, 1931.
5. Smith, E. R. B., and Cowgill, G. R.: Meat Extractives as Stimulants for Secretion of Pepsin, Proc. Soc. Exper. Biol. & Med. 30: 1284, 1933.
6. Tessieri, Iris: The Action of Peptone on the Gastric Secretion, Semana méd. 37: 1579, 1930.
7. Upham, Roy, and Spindler, F.: Studies on Humans With a New Secretagogue Meal, Am. J. Digest. Dis. 5: 721, 1939.
8. Wilhelmj, C. M., O'Brien, F. T., and Hill, F. C.: An Improved Gastric Test Meal, Am. J. Digest. Dis. & Nutrition 3: 319, 1936.

FURTHER OBSERVATIONS ON THE ROLE OF DIET IN THE ETIOLOGY AND TREATMENT OF SPONTANEOUS HYPOGLYCEMIA*

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THE manifestations of spontaneous hypoglycemia are sufficiently well recognized to require no further comment. There are apparently several causes for the syndrome, and once the diagnosis has been definitely established proper therapy cannot be instituted until the etiology is ascertained. It was assumed at first that all cases were due to an overproduction of insulin by the pancreas. This assumption led to the frequent feeding of carbohydrates to the milder cases and to surgical exploration for pancreatic tumor in the severe ones. It soon became apparent that pancreatic tumors were not present even in all of the extreme cases and that other etiologic factors must be considered. Faulty dietary habits appeared to be a likely etiologic factor in certain cases, and such a concept was supported by the fact that many patients became worse when treated with a high carbohydrate diet divided into several small meals daily. A low carbohydrate, high fat diet was proposed, therefore, as a therapeutic procedure by Clark and Greene,¹ and by Waters,² with successful results. Conn³ proposed a diet high in fat and protein, and low in carbohydrates after he observed a patient who had become worse with a high carbohydrate diet and did not improve with a high fat diet. He demonstrated that there was no appreciable hyperglycemia following such a diet and, therefore, there was no hypoglycemia. His patient was relieved of manifestations for a period of three months. Newburgh and Conn⁴ then observed six patients with spontaneous hypoglycemia and infectious hepatitis which was usually secondary to gall bladder disease. A cure was obtained in one case following removal of a diseased gall bladder. They attributed the disturbance of carbohydrate metabolism to the hepatitis.

Since the report of Clark and Greene,¹ we have observed eleven patients with spontaneous hypoglycemia due to faulty dietary habits. It was thought at first that all such cases were due to an excess carbohydrate intake until one patient who was observed in the Out-Clinic where the diagnosis was established, was admitted to the ward and given a high carbohydrate diet in an attempt to make the condition worse. On the contrary, the symptoms subsided and the glucose tolerance returned to normal. A careful dietary history then revealed that he had followed a high fat and low carbohydrate diet for several months prior to and after development of the spontaneous hypoglycemia. Since then we have

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observed two other patients who have developed their symptoms while on a high fat and low carbohydrate diet. The remaining patients have followed a high carbohydrate and low fat type of diet. The two cases here presented are typical illustrations of the two types of faulty diets which will produce spontaneous hypoglycemia. Case 1 illustrates the high carbohydrate and low fat diet as an etiologic factor, and Case 2 illustrates the low carbohydrate and high fat dietary type.

CASE 1.—A male dry cleaner, aged 32 years, entered the University Hospitals in July, 1937, because of a stone in the right lower ureter. His symptoms disappeared after spontaneous passage of the stone, but he returned in March, 1938, complaining of vague abdominal pain, constipation, palpitation, and nervousness. No organic disease was discovered and he was sent home without treatment.

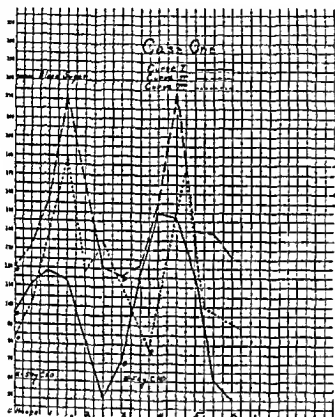


Fig. 1.—The eradication of hypoglycemia in Case 1 by a diet high in fat and low in carbohydrate, as shown by the double glucose tolerance curves.

He returned again in September, 1938, however, complaining of increasing nervousness and irritability, weakness, hunger, palpitation, fatigability, insomnia, mild constipation, slight abdominal distress, and loss of five pounds of body weight in spite of an excellent appetite. Most of these symptoms occurred two or three hours after each meal, and were severe enough to force him to stop work. Food relieved the symptoms at such times sufficiently for him to resume work, and hence he habitually ate between meals. A bedtime snack would also prevent the symptoms at night. His diet had been extremely high in carbohydrate and low in fat, primarily because of a dislike for fatty foods.

The physical examination revealed no noteworthy abnormalities. The double glucose tolerance curve (curve I, Fig. 1) was typical for hypoglycemia. After six days of a diet containing 70 Gm. of protein, 116 Gm. of carbohydrate, and 211 Gm. of fat, the double glucose tolerance curve (curve II, Fig. 1) was within normal limits and the symptoms had disappeared completely. He was discharged with a diet containing 80 Gm. of protein, 170 Gm. of carbohydrate, and 200 Gm. of fat.

He returned four months later entirely free of symptoms, and the glucose tolerance curve (curve III, Fig. 1) continued within normal limits. He was sent home with instructions to follow a diet containing 125 Gm. of protein, 200 Gm. of carbohydrate, and 250

Gm. of fat, and to return to the clinic if the symptoms reappeared. A report one year later stated that he was entirely free of symptoms.

CASE 2.—A farmer, aged 28 years, entered the University Hospitals in 1935, because of abdominal distress, nervousness, palpitation, and sweating. He had enjoyed good health until 1936, when alternating constipation and diarrhea, heartburn, water brash, vomiting, and slight generalized abdominal distress appeared. He was examined by his local physician who prescribed a bland diet. In addition, because of a dislike for carbohydrate foods, the patient reduced the carbohydrate content of his diet to a minimum. The symptoms disappeared, but several months later they returned and were accompanied by weakness, palpitation, and sweating. These symptoms occurred two to three hours after meals, and it was soon discovered that eating between meals would give relief until the next meal. The symptoms increased, however, and became so severe that he had to stop work.

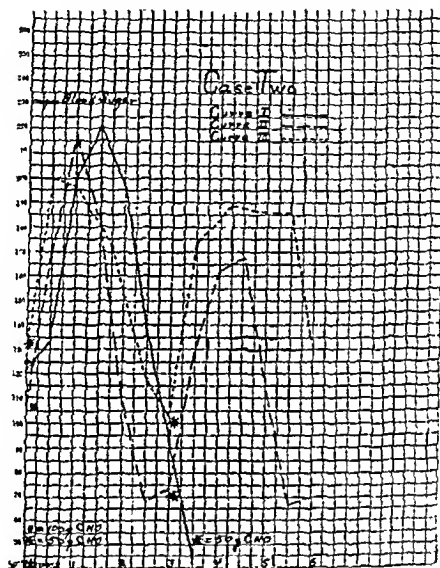


Fig. 2.—Hypoglycemia disappeared in Case 2 following a diet high in carbohydrate and low in fat, as shown by the double glucose tolerance curves.

The physical examination revealed no noteworthy abnormalities. The gastroenteric tract was normal to roentgen-ray studies. The glucose tolerance curve (curve I, Fig. 2) was typical of hypoglycemia. The patient was sent home for three weeks with instructions to follow a diet containing 500 Gm. of carbohydrate daily. He did not follow the instructions completely, but the symptoms were less when he returned and the double glucose tolerance curve (curve II, Fig. 2) was improved. He was discharged with the advice to follow the instructions and to return in three months. He returned two and one-half months later, stating that he had followed the diet, that his symptoms had completely disappeared, and that he had resumed work. The double glucose tolerance curve (curve III, Fig. 2) was greatly improved. He was instructed regarding a diet containing 90 Gm. of protein, 350 Gm. of carbohydrate, and 175 Gm. of fat before discharge and advised to return if his symptoms reappeared. A report one year later stated that he was entirely well.

COMMENT

There appear to be several causes for spontaneous hypoglycemia. Once the diagnosis is established the etiology must be ascertained before proper treatment can be instituted. The known causes for spontaneous hypoglycemia are (1) an oversecretion of insulin due to either tumor of the pancreas or hyperplasia of the

islets of Langerhans; (2) Addison's disease; (3) probably certain pituitary dysfunctions; (4) infectious hepatitis, which is usually secondary to gall bladder disease; and (5) faulty dietary habits. The syndrome can be produced by either extreme in the carbohydrate and fat content of the diet: A high carbohydrate and low fat diet or a low carbohydrate and high fat diet can produce the manifestations.

It is usually not difficult to establish the etiology in a given case. The manifestations in patients with an oversecretion of insulin are usually extreme and are not improved by dietary therapy, although they may be partially or entirely alleviated as long as adequate and frequent carbohydrate intake is continued. Addison's disease is not likely to be overlooked if its presence is once considered. The presence of pituitary dysfunction can be ascertained only when there are other manifestations of such dysfunction, and these cases are undoubtedly extremely rare. An infectious hepatitis may produce severe manifestations, and these cases are probably more common than the above-mentioned ones. A thorough study of the biliary tract is indicated in each case of spontaneous hypoglycemia, but the absence of evidence of gall bladder disease does not absolutely exclude hepatitis, nor does the presence of gall bladder disease definitely establish its presence. In patients with spontaneous hypoglycemia due to faulty dietary habits the manifestations are usually mild, but occasionally they are severe. There are many functional complaints in patients with spontaneous hypoglycemia due to faulty diets, and these complaints frequently obscure those of hypoglycemia. In fact, the functional complaints have caused most of these patients to follow the abnormal diet. The eradication of the hypoglycemic manifestations does not cure such patients, but the neurotic tendencies cannot be controlled as long as the symptoms of hypoglycemia persist. A careful dietary history is necessary, therefore, in such cases to ascertain whether or not the previous diet has been high in fat or high in carbohydrate. A high carbohydrate diet will not eradicate hypoglycemia if this type of diet has produced it, but a high fat diet will correct it. The reverse is true if a high fat diet produced the syndrome.

If the manifestations are extreme, the etiology of a given case of spontaneous hypoglycemia is most likely an infectious hepatitis or an overproduction of insulin from a pancreatic tumor. The former etiologic factor can be ascertained fairly accurately by a careful study of the biliary tract. On the other hand, the etiology is most likely an infectious hepatitis or a faulty diet if the manifestations are mild or moderately severe. The latter, in our opinion, is the most common cause for spontaneous hypoglycemia and can be uncovered by a careful dietary history and by the response to a diet which contains carbohydrate and fat in the reverse proportion to that which the patient has followed previously.

The time required for such diets to eradicate hypoglycemia in these patients varies considerably. It has required from six days to six months in our patients. The minimum duration for such dietary regimen after correction of the hypoglycemia is not known. We have assumed that the patients who respond quickly need not follow such a diet as long after correction of the hypoglycemia as those who are slower to respond. We, therefore, have advised our patients to

adhere to such diets for periods varying from three weeks to two and one-half years after the hypoglycemic manifestations have subsided. Probably such patients should be re-examined at three- to six-month intervals during therapy.

It is important that the patient be informed of the cause of the manifestations and that adequate general dietary instructions be given which will prevent a repetition of dietary mistakes after the specific dietary regime has been discontinued.

SUMMARY

The causes of spontaneous hypoglycemia have been discussed and those due to faulty diets have been emphasized. The syndrome can be produced by diets low in carbohydrate and high in fat, or by those high in carbohydrate and low in fat. Three cases due to the former and eight due to the latter have been observed, but only one case illustrative of each dietary etiology is reported. The types of corrective diets and the duration of therapy have been discussed.

REFERENCES

1. Clark, B. B., and Greene, J. A.: Effect of Low Carbohydrate Diet on Glucose Tolerance in Spontaneous Hypoglycemia, *Proc. Soc. Exper. Biol. & Med.* 32: 1459, 1935.
2. Waters, W. C., Jr.: Spontaneous Hypoglycemia: The Role of Diet in Etiology and Treatment, *South. Med. J.* 24: 249, 1931.
3. Conn, J. W.: The Advantage of a High Protein Diet in the Treatment of Spontaneous Hypoglycemia, *J. Clin. Investigation* 15: 673, 1936.
4. Conn, J. W., Newburgh, L. H., Johnston, M. W., and Sheldon, J. M.: Study of the Deranged Carbohydrate Metabolism in Chronic Infectious Hepatitis, *Arch. Int. Med.* 62: 765, 1938.

THE EFFECT OF COPPER AND IRON ON HEMOGLOBIN REGENERATION*

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THAT copper plays a role in the synthesis of hemoglobin has been conclusively demonstrated, but since it is not a part of the hemoglobin molecule, the exact mechanism of its action has not been ascertained, and the clinical implications to be drawn from animal experimentation are not entirely clear. The numerous reports concerning its effect on the anemia of experimental animals have been adequately summarized.¹⁻⁵ The present concept, in brief, is that copper acts as a catalytic agent in hemoglobin formation and aids in the mobilization and utilization of iron stored in the body as well as augmenting the effect of inorganic iron salts administered by mouth. These effects are illustrated by the fact that neither iron nor copper alone are effective in the treatment of nutritional anemia in the rat, whereas a combination of the two produces a rapid hemoglobin response. When iron has been given to an anemic rat without producing a hemoglobin response and its administration then discontinued, it has been found that copper alone will then produce rapid hemoglobin regen-

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eration by aiding in the utilization of the iron previously stored.³ The results in chronic anemia in dogs have been similar but less consistent than those observed in rats.^{6, 7}

The clinical reports on the use of copper and iron in the treatment of anemia are conflicting. An increase in the hemoglobin of infants has been reported following the use of 25 mg. of iron and 1 mg. of copper daily, and in severe nutritional anemia the hemoglobin response has been rapid. When the same amount of iron was administered alone, the response was less satisfactory.^{8, 9} This combination of copper and iron not only has been more effective than iron alone in raising the hemoglobin level but also has caused a greater gain in weight and lessened the incidence of infections among infants.¹⁰ In adults the administration of copper and iron has been reported to be effective in "secondary anemia,"¹¹ idiopathic hypochromic anemia,¹² and in anemia which accompanies the therapeutic use of malaria.¹³ Other reports have indicated that the addition of copper to an inorganic iron salt was not necessary,¹⁴ and in a study of a group of college women iron alone produced as good results as did the combination of iron and copper.¹⁵ It has been assumed in the cases in which iron alone has produced a satisfactory response that either there was no copper deficiency or there was sufficient copper obtained from the food or as a contaminant of the inorganic iron salt.

In a previous report¹⁶ concerning the effect of copper on iron metabolism in adult patients with hypochromic anemia, it was shown that the addition of copper sulfate to a relatively small dose of iron and ammonium citrates (217 to 261 mg. of elemental iron per day) led to a diminution in the amount of iron retained by the body but that hemoglobin regeneration was active in spite of the diminished iron retention. In two other patients receiving larger amounts of iron and ammonium citrates (426 and 517 mg. of iron per day) the addition of copper sulfate did not influence the retention of iron. In both groups of patients hemoglobin regeneration was active regardless of the amount of iron retained, but in neither group was the increase more rapid than that obtained with iron alone.

In an attempt to gain further information on the effect of copper on hemoglobin formation, 20 patients with mild grades of hypochromic anemia were selected for study. All were females and all were nurses, students, or hospital employees who would cooperate and who could be depended upon to take the medication regularly. One patient was found to have menorrhagia and was dropped from the study, and others dropped out after the first and second periods. The study was divided into three periods, each of six weeks' duration. During the first period the patients received 0.5 Gm (7.5 grains) of iron and ammonium citrates daily. During the second period they received the same amount of iron plus copper sulfate, and during the third period the copper was discontinued but the dose of iron and ammonium citrates was increased to 1 Gm. daily. Hemoglobin determinations by the Newcomer method, erythrocyte counts, and hematocrit determinations were made at approximately two-week intervals on all patients. Except for the initial readings, only the hemoglobin values are recorded in this communication, since these present the essential information. All medications were supplied in gelatin capsules.

RESULTS

Period I. Iron and ammonium citrates 0.5 Gm. per day (approximately 85 mg. of elemental iron). At the onset of therapy the blood hemoglobin values ranged from 8.46 to 11.57 Gm. per 100 c.c., with an average of 9.88 Gm. for the 19 patients. Following the administration of iron for forty-two days, the average gain in hemoglobin per patient was 0.41 Gm. The gain or loss for each patient is shown in Table I. The greatest increase in hemoglobin was

TABLE I

INITIAL BLOOD VALUES AND HEMOGLOBIN READING AT THE END OF EACH OF THREE PERIODS WITH INCREASE OR DECREASE DURING THAT PERIOD

CASE	INITIAL BLOOD VALUES			PERIOD I		PERIOD II		PERIOD III	
	R.B.C. MILLIONS	HEMAT. %	HB. GM.	HB. GM.	INCREASE GM.	HB. GM.	INCREASE GM.	HB. GM.	INCREASE GM.
1	4.05	34	9.32	10.07	+0.75				
2	4.19	33	9.62	9.62	0				
3	4.34	34	10.37	10.52	+0.15				
4	4.88	34	8.46	9.02	+0.56				
5	4.17	37	10.37	10.94	+0.57	10.94	0		
6	3.61	34	9.47	9.92	+0.45	9.32	-0.60		
7	4.91	40	11.57	11.15	-0.42	11.57	+0.42		
8	4.44	35	11.36	11.78	+0.42	11.99	+0.21		
9	4.35	37	10.52	10.73	+0.21	11.15	+0.42		
10	3.62	34	8.90	10.07	+1.17	10.37	+0.30		
11	4.07	36	10.37	11.57	+1.20	11.36	-0.21	10.52	-0.84
12	4.08	35	10.52	10.73	+0.21	10.94	+0.21	10.94	0
13	3.65	35	10.22	10.22	0	10.07	-0.15	11.15	+1.08
14	3.95	34	8.80	9.62	+0.82	10.37	+0.75	10.37	0
15	4.57	32	8.68	8.90	+0.22	9.77	+0.87	10.22	+0.45
16	3.67	35	9.92	9.92	0	10.52	+0.60	10.73	+0.21
17	3.82	37	10.22	10.07	-0.15	10.52	+0.45	10.94	+0.42
18	4.80	38	10.07	11.36	+1.29	10.94	-0.42	10.94	0
19	4.22	40	9.02	9.47	+0.45	10.07	+0.60	11.78	+1.71
Average gain					+0.415		+0.233		+0.336

found in Case 11, who had an initial hemoglobin value of 10.37 Gm. and during the six-week period showed a gain of 1.2 Gm. In 3 patients (Cases 2, 13, and 16) there was no hemoglobin increase, and in 2 patients (Cases 7 and 17) there was a decrease in spite of this therapy. This slow and inconsistent hemoglobin increase cannot be considered as a satisfactory response to an anti-anemic remedy, but this small dose of medicinal iron was purposely chosen so that the response would not be adequate. It was felt that if copper was of value in increasing the effectiveness of inorganic iron in hemoglobin formation, it would be manifest by an increased rate of regeneration when the copper was added to a small dose of iron. By using an amount of iron too small to be efficient, the beneficial effects of copper would not be masked by the rapid increase obtained with large doses of iron.

Period II. Iron and ammonium citrates 0.5 Gm. per day plus 3 mg. of copper as copper sulfate (ratio of Fe to Cu 28:1). Four patients discontinued treatment after the first period, leaving 15 under observation during the second period. The hemoglobin values ranged from 9.47 to 11.78 Gm., with an average of 10.43 for the 15 patients. During this period of six weeks the average gain in hemoglobin was 0.23 Gm. The greatest increase was in Case 15, with a gain of 0.87 Gm. In one patient (Case 5) there was no increase in hemoglobin, and

in 4 patients (Cases 6, 11, 13, and 18) there was a decrease. In 6 patients the hemoglobin increase was more rapid with copper and iron than it had been with iron alone; in one patient it was the same; but in the other 8 patients it gained more rapidly before the addition of copper. The average hemoglobin increase was lower than that obtained with the same amount of iron alone, so that the addition of copper did not appreciably increase the effectiveness of inorganic iron. The average hemoglobin level at the onset of treatment with copper and iron was slightly higher than when iron alone was started (10.43 Gm. as compared to 9.88 Gm.), but this is not great enough to make an appreciable difference.

Period III. Iron and ammonium citrates, 1 Gm. daily (approximately 170 mg. of elemental iron) without copper sulfate. Nine patients were followed through the third period and received twice the amount of iron previously administered. The blood hemoglobin values at the start of the period ranged from 9.77 to 11.36 mg., with an average of 10.50 Gm. The average gain in hemoglobin per patient for this six-week period was 0.34 Gm. The greatest gain was 1.71 Gm. in Case 19. Three patients failed to gain (Cases 12, 14, and 18), and one (Case 11) showed a decrease. The average hemoglobin increase was greater than that obtained with half the amount of iron plus copper sulfate and shows that the patients still have the ability to respond when iron is available in sufficient amounts.

It has been our experience with iron therapy that (in patients with mild anemias) a peak in hemoglobin regeneration is reached and thereafter there is a slight drop in the hemoglobin level even though the iron is continued. In this group of 9 patients such a peak was reached before the end of the period (in 6 instances), and a subsequent drop in hemoglobin occurred. Had the hemoglobin gain been calculated from the highest level rather than the reading at the end of six weeks, the average gain would have been 0.54 Gm. rather than 0.34 Gm.

It is possible that the greater hemoglobin increase during period III as compared to period II might be due to the copper which was stored in the body during the preceding six weeks rather than to the increased amount of iron administered. However, in another group of 12 patients with a similar hemoglobin level (10.32 Gm. per 100 c.c.) who were started immediately on 1 Gm. of iron and ammonium citrates per day, there was an average gain of 1.29 Gm. of hemoglobin during a six weeks' period. This is still greater than the gain recorded in the 9 patients during period III and indicates that the increased effect was due to the increased amount of iron rather than to the previously administered copper.

SUMMARY

The addition of copper sulfate to an inorganic iron salt did not increase the effectiveness of iron in hemoglobin regeneration in mild hypochromic anemias in the adult.

REFERENCES

1. Elvehjem, C. A.: The Biological Significance of Copper and Its Relation to Iron Metabolism, *Physiol. Rev.* 15: 471, 1935.
2. Josephs, H. W.: Studies on Iron Metabolism and the Influence of Copper, *J. Biol. Chem.* 96: 559, 1932.

3. Elvehjem, C. A., and Sherman, W. C.: The Action of Copper in Iron Metabolism, *J. Biol. Chem.* 98: 309, 1932.
4. Cook, S. F., and Spilles, N. M.: Some Factors Regulating the Utilization of Splenic Iron, *Am. J. Physiol.* 98: 626, 1931.
5. Elvehjem, C. A., and Hart E. B.: The Necessity of Copper as a Supplement to Iron for Hemoglobin Formation in the Pig, *J. Biol. Chem.* 95: 363, 1932.
6. Robscheit-Robbins, F. S., Elden, C. A., Sperry, W. M., and Whipple, G. H.: Blood Regeneration in Severe Anemia. XII. Potent Influence of Inorganic Ash of Apricots, Liver, Kidney, and Pineapple, *J. Biol. Chem.* 79: 563, 1928.
7. Elden, C. A., Sperry, W. M., Robscheit-Robbins, F. S., and Whipple, G. H.: Blood Regeneration in Severe Anemia. XIII. Influence of Certain Copper Salts Upon Hemoglobin Output, *J. Biol. Chem.* 79: 577, 1928.
8. Elvehjem, C. A., Siemers, A., and Mendenhall, D. R.: Effect of Iron and Copper Therapy on Hemoglobin Content of the Blood of Infants, *Am. J. Dis. Child.* 50: 28, 1935.
9. Elvehjem, C. A., Duckles, D., and Mendenhall, D. R.: Iron Versus Iron and Copper in the Treatment of Anemia in Infants, *Am. J. Dis. Child.* 53: 785, 1937.
10. Usher, S. J., MacDermot, P. N., and Lozinski, E.: Prophylaxis of Simple Anemia in Infancy With Iron and Copper, *Am. J. Dis. Child.* 49: 642, 1935.
11. Kropp, G. V., Goodale, R. H., and Cox, C. A.: Observations on the Use of a Copper and Iron Compound in Secondary Anemia, *M. Rec.* 150: 67, 1939.
12. Mills, E. S.: The Treatment of Idiopathic (Hypochromic) Anemia With Iron and Copper, *Canad. M. A. J.* 22: 175, 1930.
13. Schube, P. G., and Prescott, B. D.: The Effect of Copper and Iron Upon the Secondary Anemia of Therapeutic Malaria in General Paresis, *J. LAB. CLIN. MED.* 24: 346, 1939.
14. Bethell, F. H., Goldhamer, S. M., Isaacs, R., and Sturgis, C. C.: The Diagnosis and Treatment of the Iron-Deficiency Anemias, *J. A. M. A.* 103: 797, 1934.
15. Duckles, D., Willis, L., and Elvehjem, C. A.: The Treatment of Hypochromic Anemia in College Women, *J. Am. Dietet. A.* 12: 537, 1937.
16. Barer, A. P., and Fowler, W. M.: Influence of Copper and a Liver Fraction on Retention of Iron, *Arch. Int. Med.* 60: 474, 1937.

IODINE ABSORPTION BY THE SERUM OF EPILEPTIC PATIENTS ON BROMIDE THERAPY*

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BECAUSE of the differences of opinion in the literature we were prompted to investigate the iodine absorption values of the serum in a group of epileptic patients on sodium bromide therapy. An effort was made to determine whether the bromide caused any saturation of the unsaturated fatty acids of the serum, and hence be a possible explanation of the common occurrence of skin eruptions on bromide medication. The iodine absorption value was correlated with the level of serum bromide. Cholesterol studies were also done on this group of patients.

During their studies on the nature of serum antitrypsin, Jobling and Petersen⁷ showed that the ferment-inhibiting properties of the serum are due to the presence of compounds of the unsaturated fatty acids. They also showed that the iodine absorption value of a serum treated with potassium iodide falls to almost one-fifth of its original value—an effect which is probably due to the liberation of small amounts of free iodine from the potassium iodide which can then saturate the unsaturated carbon bonds. Furthermore, they concluded that the influence of iodine in removing the inhibiting action of serum shows that the inhibiting agents belong to the unsaturated group of fatty acids. From

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this was evolved the following explanation for the cause of acne in patients on bromide and iodide therapy. It was suggested that the bromine and iodine radical neutralize the unsaturated fatty acid antitryptic effect. This allows the proteolytic ferment to produce proteotoxins and thus produce the skin eruptions.

Subsequent to this Burr and Burr¹ observed that young rats restricted to fat-free diets do not thrive and develop scaldiness of the skin and tail, which could be prevented or alleviated by adding unsaturated fatty acids to the diet. They used linoleic or linolenic acid and termed them "essential fatty acids" because of this ability. In 1933 Hansen⁵ investigated the significance of the unsaturated fatty acids in cases of infantile eczema. He showed that in 21 determinations on 10 infants with eczema the amount of iodine absorbed per 100 c.c. of serum ranged between 280 and 487, with an average of 383 mg. per 100 c.c. of serum. In 18 determinations on 16 control infants the amount of iodine absorbed per 100 c.c. of serum ranged between 460 and 711, with an average of 539 mg. per 100 c.c. of serum. The average iodine number of the serum fatty acids in the eczema group was 84, and in the control group was 111. He concluded that the serum fatty acids are more saturated in cases of infantile eczema than in control infants. He⁶ also reported that in infants with intractable eczema clinical improvement was shown when oils rich in unsaturated fatty acids were administered internally for various lengths of time. This was associated with a rise to normal of the iodine numbers of the serum lipids. This could not be confirmed by Faber and Roberts,³ who could find no difference between the incidence of recovery for patients treated this way and those subjected to the usual therapeutic procedures. Taub and Zakon,⁸ working on a small series of cases of atopic dermatitis (neurodermatitis), concluded that some patients were actually worse off after taking raw linseed oil internally.

Cornbleet² is the only one who confirms Hansen's opinion. He reported uniformly good results when oils rich in unsaturated fatty acids were given to a large series of patients with chronic eczema. This was again denied in 1937 by Ginsberg, Bernstein, and Iob,⁴ who could find no characteristic abnormality of the blood lipids in patients treated, or that any specific benefit arises from feeding such patients oils containing highly unsaturated fatty acids.

METHOD

Blood was drawn in the morning on a fasting stomach. The blood was allowed to clot and the serum was removed. One cubic centimeter of serum was extracted with three parts alcohol and one part ether. When cooled, it was made up to a volume of 50 c.c. Ten cubic centimeters of this extract were used for the cholesterol determination according to Bloor's oxidative method. The remainder of the extract was used for the determination of amount of iodine absorbed per 100 c.c. of serum. The blood bromide values were determined in almost all cases on a blood specimen drawn on the same day by the Wuth colorimetric method.

The iodine absorption value of the serum was determined by the micro-method of Rosenmund and Kulnhenn, as modified by Page, Pasternack, and Burr.⁹ Yasuda's¹⁰ technique was used except that 0.01 N thiosulfate was used for titration.

In this work we took the number of milligrams of iodine absorbed per 100 c.c. of serum (after correcting for the cholesterol factor) as being quite indica-

tive of the iodine number of the total unsaturated fatty acids. We believed this tenable because practically the only substances in the serum that will absorb iodine are unsaturated fatty acids and cholesterol. In addition, the alcohol-ether mixture extracts only lipoids. Since the amount of iodine absorbed by the cholesterol was deducted in the formula, it is correct to say that N equals the number of grams of iodine absorbed by the unsaturated fatty acids in 100 c.c. of serum. It has been shown in the work of Hansen and Boyd^{6, 11} that the fatty acids are fairly constant in amount in different individuals. In Hansen's infants there was practically no difference in the fatty acid content of the serum regardless of whether they had eczema. Hansen's work clearly indicates that N parallels the iodine number of the fatty acids. We feel justified, therefore, in considering N as being indicative of the degree of unsaturation of the fatty acids in the serum. This made it possible to avoid the long and complicated procedure of actually measuring the quantity of fatty acids.

The figures for N represent the number of milligrams of iodine absorbed by the fatty acids in 100 c.c. of serum, i.e., exclusive of the cholesterol. These are not exactly comparable to those quoted from Hansen because he gave values for the amount of iodine absorbed by both cholesterol and fatty acids.

RESULTS

I. Cholesterol Values.—Cholesterol values were determined in 45 instances on 41 epileptic persons, divided into two groups: (1) those who had not started taking sodium bromide, or whose blood bromide was too low to be estimated; (2) those who had a measurable amount of bromide in the blood.

Group I—11 determinations.

The range of cholesterol was from 171 to 294 mg. per 100 c.c. of serum. The average value was 209. These limits are reasonably close to the normal cholesterol range of 160 to 250 mg. per 100 c.c. of serum.

Group II—34 determinations.

The range of cholesterol was from 132 to 368 mg. per 100 c.c. of serum. The average value was 203. There were only 8 determinations over 250, and 2 of them were on a patient with hypothyroidism. There was no significant alteration in the serum cholesterol values of epileptic persons on sodium bromide therapy.

II. Relationship of Cholesterol Values to Blood Bromide Level.—The patients were grouped into four classes according to the blood bromide level. The cholesterol values in each group were averaged with the following result:

Blood Bromide Range	Average Cholesterol in this Range
75 to 150	240 (6 cases)
151 to 200	230 (15 cases)
201 to 250	240 (8 cases)
251 to 300+	204 (5 cases)

These data indicate that the level of bromide in the blood had no material effect upon the cholesterol value. The group from 251 to 300+ mg. of sodium bromide per 100 c.c. serum had a lower average cholesterol than the others but was well within normal limits and is, therefore, not significant.

III. Relationship of Acne to Blood Bromide and Cholesterol Levels.—A survey of Table I shows distinctly that the severity of the acne is independent

of the blood bromide level. In those patients who have mild acne or seborrheic skin prior to bromide medication it was noticed that the bromide usually caused an exacerbation of the skin lesions. This was independent of the quantity of bromide ingested or of the level to which the blood bromide had been elevated. It was also noted that the age of this susceptible group was roughly in the adolescent and postadolescent period.

TABLE I

Mean iodine value is 508; median iodine value is 527.

CASE NO.	AGE	IODINE ABSORPTION VALUE	SERUM BROMIDE	SERUM CHOLESTEROL	ACNE	REMISSION RATING
1	39	698	-75	203	++	R.X.
2	42	692	150	227	+	F.R.
3	12	661	225	208	++	Re.
4	17	660	0	200	+	--
5	24	660	175	284	±	R.X.
6	52	660	220	344	++	R.X.
7	20	648	115	227	++	F.R.
8	20	597	200	163	+	R.X.
9	29	593	0	200	0	--
10	13	589	0	200	+++	--
11	15	571	210	187	+++	R.X.
12	40	569	-75	294	0	F.R.
13	50	500	185	263	+	F.R.
14	18	554	220	212	+	R.X.
15	27	552	0	212	0	--
16	30	545	250	195	+	F.R.
17	31	545	250	256	+	F.R.
18	33	533	275	208	+	I.R.
19	39	527	200	250	+++	Same case as No. 1.
20	27	526	160	250	+	Re.
21	15	508	190	297	+	R.X.
22	9	507	165	186	0	I.R.
23	35	503	275	193	+++	F.R.
24	60	495	190	272	0	F.R.
25	19	489	260	227	0	F.R.
26	7	467	250	368	0	I.R.
27	28	464	110	234	0	F.R.
28	17	464	220	188	+	F.R.
29	20	457	160	227	0	R.X.
30	40	438	135	321	+	I.R.
31	50	397	110	208	0	F.R.
32	16	394	0	171	++	--
33	22	371	160	132	++	R.X.
34	52	362	160	216	0	F.R.
35	9	330	300	208	0	F.R.
36	17	314	170	182	++	R.X.
37	17	277	235	150	++	Same as No. 36.
38	21	108	300+	184	+++	R.X.

It is also apparent that there is no relationship between the amount of cholesterol in the blood and the severity of the acne. The patients were classified into four groups, according to the severity of the acne. The average cholesterol value for each group is indicated below:

Acne
0 (No acne)
+ (Mild)
++ (Moderate)
+++ (Severe)

Average Cholesterol

234 (13 cases)
242 (16 cases)
200 (8 cases)
203 (5 cases)

From these data there appears to be some difference between the two extremes of no acne and +++ acne. This difference is of no significance clinically, since both extremes are in the normal range.

IV. Relationship of Iodine Absorption to the Blood Bromide Level.—In 34 patients the iodine absorption value of the fatty acids of the serum was measured on the same specimen of blood as was the blood bromide. These patients had been on sodium bromide for varying periods of time and their blood bromide levels varied from -75 to 300+ mg. of sodium bromide per 100 c.c. of serum. One can see that there is very little relationship between the amount of iodine absorbed by the fatty acids in 100 c.c. of serum and the blood bromide level. A perusal of Table I reveals a range of iodine values from 698 down to 108. The blood bromide values ranged from -75 to 300+. In only very occasional instances was a high iodine value correlated with a low blood bromide value. There were many more instances in which such a correlation did not exist.

V. Relationship of Iodine Absorption Value to the Presence of Acne.—A general survey of Table I shows that there are cases of acne distributed over the whole range of iodine absorption values from 108 to 698. This would, therefore, appear to yield a negative result. That is to say, it is not probable that the bromide taken orally serves to saturate any unsaturated fatty acids and thus inactivate the antitryptic ferments. However, if one derives an average iodine absorption value (508), it is apparent that there are 18 cases of acne above this average and only 8 cases below. This would seem to indicate that there is a general tendency for acne to occur more readily in patients with a high iodine absorption value than in those with a low value. In other words, it appears that the higher the level of unsaturated fatty acids the easier it is for acne to occur. This is exactly opposed to the original theory of Jobling and Petersen.

Table I shows that there are 20 patients in the scale above the average iodine absorption value and 17 below the average. Of the 20 above the average there are 13 under 30 years of age and 8 under 20 years of age, the average age being 27 years. Of the 17 below the average there are 12 under 30 years of age and 9 under 20 years of age, the average age being 26 years. Therefore, it can be stated that the general tendency for greater prevalence of acne in the group with higher iodine absorption values is not determined by the difference in ages of the two groups.

VI. Relationship of Iodine Absorption Values to Remissions of Convulsions.—In this group of patients 31 were followed and classified according to response to treatment.

1. Initial Remission. There were four patients who had a remission over six months of all spells at the onset of treatment. The iodine absorption value ranged from 438 to 533, with an average of 486.

2. Final Remission. Fourteen patients eventually had a remission of all spells lasting six months or more. The range of iodine absorption value was 330 to 692, with an average of 504.

3. Remissions and Exacerbations. Eleven patients showed improvement by remissions of all spells for periods of two to three months alternating with exacerbations of spells. The range of iodine absorption values was from 108 to 660, with an average value of 484.

4. Recalcitrant. In this group of two patients no improvement occurred. The iodine absorption values were 661 and 526, with an average of 593. The group was too small to be of significance.

The values in the first three groups are too close to be of any significance. There is no correlation between iodine absorption value and response to therapy with sodium bromide.

SUMMARY AND CONCLUSIONS

1. The cholesterol values in 41 patients with epilepsy revealed no significant deviation from normal prior to or during therapy with sodium bromide.
2. There was no correlation between the blood bromide level and serum cholesterol in patients on bromide therapy.
3. Acne may occur in patients on bromide therapy regardless of the blood bromide level or serum cholesterol level. Those patients with acneic or seborrheic skin were more prone to an exacerbation of their skin lesions.
4. The iodine absorption value found in our study ranged from 108 to 698, with an average of 508. There was no significant relationship between the blood bromide level and the amount of iodine absorbed by the blood serum.
5. There was no definite correlation between the iodine absorption value and the presence or severity of acne. We did not find that the acne of epileptic patients on bromide therapy was associated with a low level of unsaturation of fatty acids.
6. The response to treatment with sodium bromide was not dependent on the iodine absorption value of the blood.

REFERENCES

1. Burr, G. O., and Burr, M. M.: New Deficiency Disease Produced by Rigid Exclusion of Fat From Diet, *J. Biol. Chem.* 82: 345, 1929.
2. Cornbleet, T.: Use of Maise Oil (Unsaturated Fatty Acids) in Treatment of Eczema, Preliminary Report, *Arch. Dermat. & Syph.* 31: 224, 1935.
3. Faber, H. K., and Roberts, D. B.: Studies in Infantile Allergic Eczema, Serum Lipids With Special Reference to Saturation of Fatty Acids, *J. Pediat.* 6: 490, 1935.
4. Ginsberg, J. E., Bernstein, C., Jr., and Ioh, I. V.: Effect of Oils Containing Unsaturated Fatty Acids on Patients With Dermatitis, With Brief Report of Experimental Study in Guinea Pigs, *Arch. Dermat. & Syph.* 36: 1033, 1937.
5. Hansen, A. E.: Study of Iodine Number of Serum of Fatty Acids in Infantile Eczema, *Proc. Soc. Exper. Biol. & Med.* 30: 1198, 1933.
6. Hansen, A. E.: Serum Lipid Changes and Therapeutic Effects of Various Oils in Infantile Eczema, *Proc. Soc. Exper. Biol. & Med.* 31: 160, 1933.
7. Jobling, J. W., and Petersen, W.: The Nature of Serum Antitrypsin, Studies on Ferment Action, *J. Exper. Med.* 19: 459, 1914.
8. Taub, S. J., and Zakon, S. J.: Use of Unsaturated Fatty Acids in Treatment of Eczema (Atopic Dermatitis, Neurodermatitis), *J. A. M. A.* 105: 1675, 1935.
9. Page, I. H., Pasternack, L., and Burt, M. L.: Über den Transport von Fetten und Lipoiden durch Blut nach Oleingabe, *Biochem. Ztschr.* 223: 445, 1930.
10. Yasuda, M.: Determination of Iodine Number of Lipid, *J. Biol. Chem.* 94: 401, 1931.
11. Boyd, E. M.: Lipid Analysis of Blood Plasma in Normal Young Women by Microoxidative Methods, *J. Biol. Chem.* 101: 323, 1933.

THE STUDY OF ACETYLCHOLINE IN URINE

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ACETYLCHOLINE is an organic nitrogenous substance found physiologically in very small amounts in every body cell. It acts as a chemical transmitter of nervous impulses from the motor nerve endings to the muscle end plates.

Elliot was the first to suggest that automatic nerves transmit their impulse at their nerve endings by releasing some specific chemical substance, and Loewi later showed that parasympathetic effects are transmitted by release of acetylcholine. It was found that two chief specific substances were liberated by the nerve fibers: acetylcholine (unstable ester of choline) and an adrenalin-like compound.

Sir Henry Dale called the nerve fibers that liberate the adrenaline-like compound at their terminals "adrenergic" and those that liberate the acetylcholine "cholinergic." Cholinergic fibers are (1) preganglionic fibers of the whole sympathetic system, (2) postganglionic fibers of the parasympathetic system, and (3) motor fibers of the voluntary muscle.

When a preganglionic impulse arrives at the synapse, very small amounts of acetylcholine are liberated to play an important part in transmitting the impulse to the autonomic ganglionic cell and to influence the action of the muscle and gland cells. Feldberg and Dale demonstrated that some postganglionic fibers of the true sympathetic system transmit their effects by means of acetylcholine. Dale thinks that the action of acetylcholine must be on the effector cells and not on the nerve endings. It seems that the transmitting substance is held inactive and is released when the nerve impulse arrives.

Ergehart (1931) showed that this depot of the transmitting substance depends for its maintenance on the integrity of the nerve endings and that it disappears or becomes depleted when the nerve fibers start to degenerate.

Mott and Halliburton were unable to find the choline or its unstable ester in urine with their chemical method. Guggenheim and Loeffler described a biological method by which they were able to extract acetylcholine from urine in the smallest negligible amounts. After numerous experiments they found that a 1:1,000,000,000 solution of synthetic acetylcholine will produce minimal peristaltic movements on the small intestine of a guinea pig, and that the amount of urine necessary to produce same minimal peristalsis should contain 0.000.001 mg. acetylcholine.

Method.—From a twenty-four-hour urine specimen 2 c.c. of urine were collected, evaporated, treated with alcohol and acetylchloride, neutralized, and

finally diluted up to an amount of 100 c.e. This solution was then examined in very small increasing amounts on the small intestine of a guinea pig, and the slightest peristaltic movement was carefully recorded.



Fig. 1.—Bronchopneumonia.

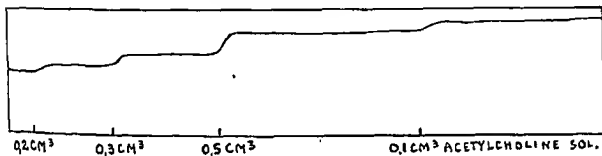


Fig. 2.—Locomotor ataxia.



Fig. 3.—Myasthenia gravis.

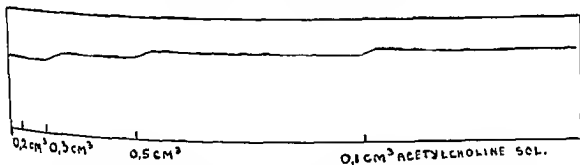


Fig. 4—Myasthenia gravis (after treatment with prostigmine).

Two cubic centimeters of urine were collected from a twenty-four-hour specimen (800 c.e.) and treated by the above-mentioned method. Choline was transformed into acetylcholine, neutralized, diluted to 100 c.e., and the resulting solution examined on the intestine of the guinea pig (Fig. 1). Two-tenths cubic centimeter of the solution did not record any peristaltic movement; 0.3 c.e. of the solution recorded minimal peristalsis; 0.5 c.e. of the solution recorded strong peristalsis; and 0.1 c.e. of acetylcholine solution that contains 0.000.001 mg. of acetylcholine recorded the same peristaltic movements as 0.3 c.e. of the examining solution, which contains the same amount of 0.000.001 mg. of acetylcholine. The whole amount (100 c.e.) of the examining solution contains $\frac{0.0001}{0.3}$

or 0.00033 mg. of acetylcholine. As 100 c.c. of the examining solution was prepared from 2 c.c. of a twenty-four-hour urine specimen (800 c.c.), the amount of acetylcholine in the whole daily specimen will be: $0.0003 \times \frac{800}{2} = 0.132$ mg

Making a study with the foregoing method, I extracted choline in the form of acetylcholine from the urine of 120 patients. In patients suffering from nephritis, arteriosclerosis, and vitium cordis, acetylcholine was found in very small amounts in urine. In five patients with myasthenia gravis treated with prostigmine, acetylcholine was eliminated in urine in very negligible amounts (0.016 to 0.100 mg.).

It was very interesting to find that choline and acetylcholine were eliminated in larger amounts in the urine of patients suffering from parasymphilitic diseases of the brain and spinal cord. In six persons with locomotor ataxia and one with general paresis with positive Wassermann test, acetylcholine was found in urine in very large amounts. In three patients with myasthenia gravis acetylcholine was eliminated in urine in surprisingly large amounts of 0.350 mg., 0.450 mg., and 0.500 mg. (in a twenty-four-hour urine specimen).

CONCLUSION

From my experiments it is evident that choline and its unstable ester "acetylcholine" stored in the nerve fibers become depleted and are eliminated in the urine in unusually large amounts in patients suffering from parasymphilitic diseases of the brain and spinal cord.

It seems that in myasthenia gravis accelerated destructive action of choline esterase is inhibited by the administration of prostigmine and the elimination of the substance in the urine is greatly decreased.

REFERENCES

1. Dale, Sir Henry: Chemical Transmission of the Effects of Nerve Impulses, Brit. M. J. p. 835, May 12, 1934.
2. Dale, Sir Henry: Pharmacology and Nerve-Endings, Proc. Roy. Soc. Med. 28: 319, 1935.
3. Loewi, Otto: Ueber humorale Uebertragbarkeit und Herznervenzirkung, Pflüger's Arch. 199: 239, 1921.
4. Loewi, Otto: Chemical Transmission of Nervous Impulse, Proc. Roy. Soc., London, s.B. 118: 299, 1935.
5. Dale, H., Feldberg, W., and Vogt, M.: Release of Acetylcholine at Voluntary Motor Nerve Endings, J. Physiol. 86: 353, 1936.
6. von Guggenheim, O., and Loeffler, W.: Ueber das Vorkommen und das Schicksal des Cholins im Tierkörper, Biochem. Ztschr. 74: 208, 1916.
7. Matanovitch, M.: Cholingehalt des Harnes unter pathologischen Bedingungen, Med. Clinie Basel, Dec. 28, 1918.
8. Pritchard, E. A. Blake: Prostigmin in the Treatment of Myasthenia Gravis, Lancet 1: 434, 1935.

CHOLINESTERASE DETERMINATIONS*

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NUMEROUS methods have been proposed and used for the estimation of the cholinesterase content of blood or tissues. Their variety has resulted from most investigators in the field making an arbitrary choice with regard to one or more of the several variables that affect cholinesterase activity. The methods fall into two classes, depending upon whether bio-assay is used to measure residual acetylcholine after enzyme action has occurred, or whether the acetic acid produced is estimated by a suitable chemical method.

The two classes of methods differ in some important respects. The bio-assay methods are usually carried out at low substrate concentrations, the reaction periods are short (often only a few seconds), the methods are laborious, and the precision of estimation of extent of hydrolysis per unit time is low. However, bio-assay methods may provide the nearest laboratory approach to the study of enzyme kinetics under physiologic conditions. The chemical methods are limited by their relative insensitivity to use of high substrate concentrations, but are capable of much greater precision. The flexibility and simplicity of chemical methods encouraged us to investigate the conditions under which they provide a satisfactory measure of the cholinesterase activity of blood.

A study of the cholinesterase activity of human blood preparations was reported¹ in which we were able to show that there are at least two enzymes possessing such activity in blood. One appears in the serum and the other in the cells, and the latter may be studied with solutions or suspensions of cells after centrifuging then washing. The effects of several variables affecting cholinesterase estimation techniques were also investigated. The present paper applies these studies to the problem of practical use of this type of assay and indicates the clinical situations in which cholinesterase determinations have given interesting results.

Over a wide range of conditions whole blood activity was found to be the sum of the separate activities of the cell and serum enzymes.¹ The cell enzyme activity markedly predominates over that of the serum as the substrate concentration is diminished to about that at which the enzymes must act in the body. The cell enzyme is inhibited by excess acetylcholine substrate, promoted by sodium ion in physiologic salt solution, and little affected by pH changes of the solution. The serum enzyme activity increases approximately with the substrate concentration up to 0.01 millimolar, and no inhibition is noted at higher concentrations.²⁻⁵ The serum enzyme has a pH optimum⁶ at about 8.0 and is inhibited only slightly by 0.85 per cent sodium chloride concentration.

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The influence of substrate concentration on rate and the inhibition of both enzymes by choline ion make it desirable to perform titrations at high substrate concentrations. The standard conditions that have been selected for the present titration method allow it to be readily applied to serum, cell, or whole blood samples with a high degree of precision.

METHOD

Blood for whole blood titrations is drawn from finger or ear prick into calibrated 0.2 ml. pipettes and immediately rinsed into 4.8 ml. of water containing 1:100,000 phenylmercuric acetate as a preservative. It may be stored on ice for a week or more before testing. For separated serum and cell titrations the technique already described¹ is satisfactory, or blood may be drawn as above, rinsed into 4.8 ml. of saline, centrifuged, and the cells washed once with saline, then laked in water to make 5 ml. The difference in sodium chloride content of the two fractions has only a slight effect on the serum activity (decrease of about 10 per cent). Possibly a better method would be to do all titrations at a final sodium chloride content of 0.85 per cent, but unfortunately the important influence of salts on the enzymes was not recognized before we had obtained our data on normal values of whole blood, serum, and cells.^{1,2} Since the values obtained can in any case have only comparative significance (see Discussion), it has not seemed worth while to repeat our work.

The titration is performed with the aid of a commercial glass electrode pH meter (Beckman model G). A test tube of 40 ml. capacity placed in a bath at 37° C., or jacketed and warmed with circulating water from a thermostat, serves as the reaction vessel. The contents are stirred by hand with a large glass bulb blown on the end of a piece of narrow tubing. The stirrer occupies 10 ml. of the volume. The electrodes are immersed in the reaction mixture to a depth of about one inch and kept there several minutes before taking readings in order to bring their contents to temperature equilibrium. It is necessary to use an excess of solid potassium chloride in the calomel half cell and to rerinse the liquid junction after temperature equilibrium is reached to keep the bridge solution saturated throughout. It is convenient to connect the electrodes to the pH meter by means of the special cable which is available. Standardized 0.02 normal sodium hydroxide is added as required from a 5 ml. burette with a capillary tip immersed in the reaction solution. A stock 0.02 molar acetylcholine solution is used which contains 5.46 Gm. of acetylcholine iodide in a liter of distilled water and is adjusted to pH 4.0 by the addition of 0.1 normal hydrochloric acid. This stock solution is kept in the dark in a tightly stoppered pyrex flask and is stable for three months or more.

The enzyme solution (5 ml.) and the acetylcholine iodide stock solution (20 ml.), separately warmed, are mixed in the reaction vessel and adjusted to pH 7.40. The burette is read and the pH is kept within 7.30 and 7.50 by small additions of standardized alkali. The time required for each 0.1 or 0.2 ml. increment of alkali is recorded to the nearest second. These should be constant within the reading errors. After about ten minutes the precision of estimation should be better than ± 2 per cent.

The amount of alkali used per twenty minutes⁸ is calculated, and a blank of 0.085 ml. is subtracted from this value to allow for the alkaline hydrolysis. The result is corrected for any pipette error to obtain the index of activity or "rate."

NORMAL VALUES

These data have been presented previously^{1, 7} and are collected here for convenient reference. In Tables I and II are shown the results of a number of individual determinations made on normal persons, *s* being the estimate of the standard deviation of their distribution.¹⁸ The data of Table I are grouped to permit an analysis of age or sex differences, and it is to be noted that no significant age or sex differences were found. In Table II repeated determinations on four normal persons are tabulated, and the data show that change of enzyme in a single person with time is confined to a range of variation only one-third of the variation from person to person.

In Table III is shown the distribution of enzyme of whole blood into serum and cell fractions and the ratio of serum to whole blood enzyme activities.

DISCUSSION

Perhaps more important than the method itself are two questions regarding the results which may be obtained: first, when may they be expected to be profitable? and second, what significance can be attributed to them? As an attempt to answer the first question, we have appended a summary of the literature on blood cholinesterase determinations of clinical and physiologic interest in the light of present knowledge of these enzymes. For the second, there are certain considerations that may assist the clinician in deciding what his results mean.

It is clear that present technique and knowledge do not permit physiologic interpretation of the results of blood or blood fraction assay. Other tissues are known to possess active enzymes, which have not been clearly characterized, and acetylcholine is readily diffusible. The only study of acetylcholine-cholinesterase physiology with which we are acquainted that seems reasonably free of critical objection is that of Glick,⁹ who worked, over a considerable range of conditions, with tissue from the superior cervical ganglion of the cat and confined his conclusions to the same organ. Significance of results must, therefore, be based on a comparison with normal values, or with a normal level established for the person studied in case it is desired to evaluate the results of therapy.

For the purpose of such comparison it has seemed to us that whole blood titrations have practically the same utility as blood fraction titrations. Under our conditions each enzyme contributes nearly the same share of the whole blood value. The probability of encountering compensatory changes in both enzymes seems negligible in view of the considerable differences in their behavior and of their demonstrated independence.

A second important consideration is occasioned by the relatively wide range of activities we, as well as others, found in ostensibly normal persons. The extremes in rates we found in about 100 assays of normal whole bloods were 0.500 to 1.068. If the rates are normally distributed about a mean of

TABLE I
TITRATIONS OF NORMAL PERSONS

PERSON	MALES AGE	RATE	PERSON	FEMALES AGE	RATE
E. V.	14	0.501	V. K.	26	0.792
B. W.	34	0.947	M. K.	26	0.748
C. B.	29	0.846	M. A.	32	0.747
C. C.	24	0.670	C. F.	24	0.716
R. W.	30	0.821	L. F.	23	0.735
D. F.	25	0.700	P. G.	22	0.542
Mean of group 0.797			F. B.	25	0.633
G. A.	36	0.780	R. L.	24	0.805
H. M.	40	0.902	Mean of group 0.714		
G. P.	46	0.876	E. J.	38	0.714
F. K.	36	0.633	G. W.	35	0.895
P. K.	35	0.724	C. S.	45	0.986
Mean of group 0.783			S. R.	58	0.803
G. M.	66	0.554	Y. R.	66	0.919
S. M.	52	0.934	Mean of females 0.772		
Mean of males 0.761			s 0.118		
s 0.144			Mean of normals 0.766		
			s 0.129		

TABLE II
TEMPORAL VARIATION OF ENZYME

BLEEDING DATE	B. W. (MALE) AGE 34	E. J. (FEMALE) AGE 38	V. K. (FEMALE) AGE 26	M. K. (FEMALE) AGE 26
9/21/36	0.865			0.703
10/ 5/36	0.967	0.736	0.785	0.735
10/ 6/36	0.918	0.714	0.736	0.763
10/ 7/36	0.971	0.687	0.838	0.724
10/ 9/36	0.910	0.698	0.825	0.737
10/10/36	1.000		0.788	0.805
10/13/36	0.968	0.810	0.793	0.710
10/20/36	0.915	0.705	0.765	0.814
10/27/36	0.981	0.702	0.822	0.720
11/ 3/36	0.911	0.736	0.754	0.746
11/13/36	0.931		0.754	0.750
11/25/36	0.960	0.704	0.803	0.739
12/14/36			0.834	
12/17/36	0.965	0.652		0.777
1/30/37	0.994	0.707	0.805	
Means	0.9469	0.7137	0.7923	0.7479
s values	0.034	0.038	0.048	0.044

TABLE III
DISTRIBUTION OF ENZYME IN BLOOD

SUBJECT	FROM 0.2 ML. BLOOD		SERUM CELLS	WHOLE BLOOD 0.2 ML.	RATIO SERUM/BLOOD
	SERUM	CELLS			
Males					
G. A.	0.50	0.30	0.80	0.80	0.62
H. M.	0.55	0.31	0.86	0.90	0.61
C. B.	0.47	0.35	0.82	0.84	0.56
G. P.	0.49	0.36	0.85	0.87	0.56
Females					
M. K.	0.43	0.34	0.77	0.74	0.58
V. K.	0.42	0.35	0.77	0.83	0.51
M. B.	0.39	0.28	0.67	0.65	0.60
Averages	0.46	0.33	0.79	0.80	0.53

0.76, with a standard deviation of 0.13, these limits would be expected to be equaled or exceeded about three times in 100 trials. Corresponding range limits for one time in 1,000, 100, and 20 are 0.33 to 1.19, 0.42 to 1.10, and 0.50 to 1.02, respectively.

These figures enable one to make an estimate of the possible significance of a single individual determination. For example, in assays on ten patients hospitalized for various conditions (unpublished data obtained through the cooperation of Dr. S. R. Baker of the Cedars of Lebanon Hospital, Los Angeles), the three lowest and the two highest values were 0.43 (coronary artery disease), 0.46 (spinal tuberculosis), 0.50 (subacute bacterial endocarditis), 1.04 (rheumatic heart disease), and 1.06 (hyperthyroidism). None of these can be considered individually significant or of diagnostic value, but they suggest that studies of these conditions might be of interest, and, indeed, in tuberculosis and in hyperthyroidism significant changes of activity have been demonstrated (see below) by comparing the average from a number of persons so afflicted with an average for normals. The range of variation of average values to be expected is much smaller than that for individual determinations and may be made as small as is desired by including a sufficient number of individuals in the groups. So far as we are aware, there is today no condition known for which the assay of blood cholinesterases can be expected to give individual results of consistent diagnostic value. It appears, however, that the possible prognostic value of individual determinations has not been explored.

CHOLINESTERASE IN DISEASE AND OTHER CONDITIONS

Ginsberg, Kohn, and Necheles¹⁰ reviewed much of the literature on blood cholinesterase determinations and came to the conclusion that a definite change had been demonstrated only in tuberculosis. However, it appears certain that the function of the (cholinergic) motor nerves is disturbed in myasthenia gravis. Walker¹¹ found that administration of cholinesterase inhibitors produces prompt but temporary relief of the symptoms of the disease. McGeorge¹² observed that the period of symptomatic improvement after giving prostigmine coincides with the period of decreased activity of the serum enzyme, as determined by the Stedman gasometric method. Fraser, McGeorge, and Murphy¹³ found that large doses of choline esters, including acetylcholine itself (given as the chloride), produced a delayed, prolonged period of relief, which they explained by postulating the utilization of some of the acetylcholine in formation of the acetylcholine precursor in the nervous tissue, since choline ion, with or without acetate, produced no such effect. Ingvarsson¹⁴ showed that a single large dose of acetylcholine iodide does not alter the activity of cholinesterase in whole blood, as determined by a modification of the Galehr and Plattner¹⁵ bio-assay method. Hall and Ettinger¹⁶ found that in dogs repeated large daily doses produced no change in serum enzyme.

With regard to the cholinesterases in the blood of myasthenic persons, there is a considerable divergence of results. Stedman and Russell¹⁷ demonstrated conclusively that, when estimated by Stedman's gasometric method, there is a significant decrease in the serum cholinesterase, while the corpuscle cholinesterase is normal in amount, as compared with normal individuals.

Fisher's "t" test¹⁸ applied to the difference between the arithmetic averages of the values of serum cholinesterase reported for 12 myasthenic and nine normal individuals, shows that a difference of this magnitude would have been obtained by accident only about one time in 50 if myasthenic persons were really no different in this respect from normal individuals (our own calculation). Two myasthenic persons, followed for a considerable period of time, showed a marked drop in blood cell cholinesterase values after spontaneous remissions of symptoms.

McGeorge's¹² observation that in three myasthenic persons the serum enzyme, measured by the Stedman titration method, was within the normal range, cannot be taken as a contradiction of the results of Stedman and Russell, since the values reported by the latter for normal and myasthenic persons overlapped. If Stedman and Russell's figures are representative, the number of individuals they measured is about as few as could be relied upon to show a significant difference. On the other hand, Hicks and MacKay¹⁹ found very large increases in serum cholinesterase in three myasthenic persons as compared with 20 normal persons. However, they failed to present data to show that the reliability and reproducibility of the bio-assay method which they used is adequate to demonstrate with certainty differences of the magnitude they found, especially since practically the whole of the discrimination depended upon differences in the rates of hydrolysis within the first five seconds of mixing the reagents. Furthermore, they used prostigmine to stop the reaction after it had proceeded for the desired time intervals, without presenting experimental justification for their assumption that the prostigmine acts practically instantaneously. Easson and Stedman²⁰ have shown that the reaction between this same inhibitor and an enzyme derived from horse serum by the Stedman and Stedman²¹ purification method requires several hours to reach equilibrium. Matthes²² also indicated that appreciable time was required to reach equilibrium in the reaction of eserine with whole blood.

Hicks and MacKay accounted for the difference between their results and those of Stedman and Russell by pointing out that their measurements were made under different conditions from those of Stedman and Russell. However, our experiments on the behavior of human serum enzyme with changes of pH and of substrate concentration show that this explanation is probably not valid, and that, should the observations of Hicks and MacKay be capable of critical verification, it will be a strong indication that the cholinesterase of myopathic sera differs qualitatively from that of normal serum.

Corkill and Ennor²³ made measurements of the total activity of whole blood of two myasthenic persons as compared with a group of normal persons using a bio-assay method to estimate the residual acetylcholine ion concentration after the enzyme had acted for twenty seconds and had been stopped by the addition of trichloroacetic acid (Galehr and Plattner,¹⁵ and Matthes²²). The myasthenic bloods fell well within the normal range of activity. This corroborates the findings of Stedman and Russell¹⁷ that the blood cell enzyme is within the normal range, since the measurements were made at a concentration of acetylcholine ion (approximately 0.0005 molar) for which we have found that serum enzyme contributes but little to the activity of whole blood.

Russell and Stedman²⁴ observed that the serum enzyme was low, in one patient with myotonia congenita, while the blood cell enzyme was normal in amount. Hicks and MacKay¹⁹ found five myotonia congenita patients to have a low serum enzyme. The figures available so far cannot be considered more than suggestive evidence of a changed cholinesterase situation in this condition.

In hyperthyroidism Antopol, Tuchman, and Selifrin²⁵ have shown that the cholinesterase of serum is markedly increased. A group of treated patients showed lower values. The same authors²⁶ have also published data showing serum values to be lowered in jaundice and biliary disease, and promise data showing decreased enzyme activity in several other conditions. They attribute the lowered values in jaundice to inhibition by bile salts.²⁷ However, their suggestion that the altered serum esterase may be related to nervous function ("sympathicotonicity or vagotonicity") must be considered at present as purely speculative, in view of the fact that no correlation between serum, blood cell, and tissue enzyme activity has been demonstrated. The same considerations apply to the observations of Tod and Jones^{28, 29} that the serum enzyme is significantly altered in certain mental states.

Investigators have been unable to find changes in the cholinesterases in a number of other disease conditions and physiologic states. Altenburger³⁰ found normal values in whole blood or spinal fluid of patients with epilepsy and with Parkinsonism, using a bio-assay method. We also found no change in titration values under our standard conditions of whole blood in a group of eight Parkinsonism patients under the care of Dr. E. Ziskind at the Los Angeles General Hospital (unpublished data). Hall and Lucas³¹ could find no correlation between serum cholinesterase (measured by a modification of the Stedman titration procedure) and age, sex, weight, exercise, fatigue, diet, blood pressure, resting heart rate, hemoglobin, and blood cell counts, or in women during menstruation or pregnancy in 40 normal and 162 hospital patients. Among the hospital patients they could find no correlation with the disease represented. Milhorat³² made a series of measurements of serum enzyme and arrived at similar conclusions. Obviously, these investigators may have overlooked significant alterations in total blood activity, because they did not include blood cell activity in their measurements.

We have been unable to find any shift in whole blood enzymes in allergic individuals or in asthmatic persons during attacks.⁷

We studied the effect of exercise upon cholinesterase content of the blood. It is known that both the motor nerve mechanism and the reflex vagus inhibition of the heart resulting from blood pressure rise act by acetylcholine mediation, and it seemed not unlikely that the increase of metabolism and blood pressure consequent to violent exercise might be reflected in changes of blood cholinesterases. Total blood cholinesterase activity measurements were made with our standard method before exercise, fifteen minutes after exercise (the time when blood lactic acid is maximal, according to Long and Lupton³³), and several hours later. The exercise was brief and sufficiently vigorous to more than double the heart rates in two normal adult males. No shifts beyond the limit of error of the method were found (unpublished).

The experiments of Manning, Lang, and Hall³⁴ on the inhibition of serum cholinesterase by eserine are interpreted by them as indicating that the ac-

cepted theory which attributes the physiologic action of eserine wholly to its inhibitory action on cholinesterase may be in error. This cannot be taken to prove their point, since Ammon and Voss³⁵ found that the cholinesterases of whole blood do not parallel serum enzyme in their inhibition by eserine. Even if experiments with whole blood enzymes were found to give similar results there would be no certainty that the enzyme of the tissues was likewise affected.

Dikshit and Mahal³⁶ demonstrated that the enzymes of whole blood are markedly affected by infection with *B. pestis* or vaccination with plague vaccine, which they interpret as a response to the toxemia and suggest that it may account for the muscular weakness of toxemias. They measured the enzymes both by a bio-assay method at low acetylcholine ion concentrations and by the Stedman gasometric procedure.³⁷ They found the agreement between the two methods to be improved by decreasing the substrate concentration in the latter method. From our work it would appear that this is a reflection of the increasingly predominant activity of the blood cell enzyme at lower substrate concentrations. The data suggest that the changes they observed were greater for the cell than the serum enzymes.

Other reports, whose expressed or implied conclusions depend on a parallelism in behavior between serum and blood cell or tissue enzymes, are the work of Glick and Antopol³⁸ on inhibition of cholinesterase activity by vitamin B₁, and of Minot³⁹ on the influence of toxic doses of guanidine on dog serum cholinesterase. Govaerts, Caubier, and van Dooren⁴⁰ concluded that varying degrees of individual sensitivity to acetylcholine were not dependent on differences in rate of destruction of the drug, because such differences could not be found in the plasmas. Ingvarsson's¹⁴ study with whole blood makes this conclusion appear more probable, but it should be noted that the conditions selected for esterase assay were far removed from those under which the enzyme acts *in vivo*, and may have prevented the detection of significant differences, even if one assumes that destruction in the blood stream is the only factor other than inequality of response that may affect sensitivity.

REFERENCES

1. Alles, G. A., and Hawes, R. C.: Cholinesterases in the Blood of Man, *J. Biol. Chem.* 133: 375, 1940.
2. Easson, L. H., and Stedman, E.: Absolute Activity of Cholinesterase, *Proc. Roy. Soc., London*, s.B. 121: 142, 1936.
3. Clark, A. J., Raventos, J., Stedman, E., and Stedman, E.: Kinetics of Cholinesterase, *Quart. J. Exper. Physiol.* 28: 77, 1938.
4. Roepke, M. H.: A Study of Cholinesterase, *J. Pharmacol. & Exper. Therap.* 59: 264, 1937.
5. Ziff, M., Jahn, F. P., and Renshaw, R. R.: The Acetylcholine-Cholinesterase System, *J. Am. Chem. Soc.* 60: 178, 1938.
6. Glick, D.: Studies on Enzymatic Histochemistry. XXV. A Micro Method for Determination of Choline Esterase, and the Activity-pH Relationship of This Enzyme, *J. Gen. Physiol.* 21: 289, 1938.
7. Hawes, R. C., and Alles, G. A.: Cholinesterase in the Blood of Allergic Patients, *J. Allergy* 12: 1, 1940.
8. Stedman, E., Stedman, E., and Easson, L. H.: An Enzyme Present in the Blood Serum of the Horse, *Biochem. J.* 26: 2056, 1932.
9. Glick, D.: Choline Esterase and the Theory of Chemical Mediation of Nerve Impulses, *J. Gen. Physiol.* 21: 431, 1938.
10. Ginsberg, R., Kohn, R., and Necheles, H.: Studies on the Blood Esterase, *Am. J. Digest. Dis. & Nutrition* 4: 154, 1937.
11. Walker, M. B.: Case Showing Effect of Prostigmine in Myasthenia Gravis, *Proc. Roy. Soc. Med.* 28: 759, 1935.

12. McGeorge, M.: Cholinesterase Activity in Disease, With Special Reference to Myasthenia Gravis, *Lancet* 1: 69, 1937.
13. Fraser, F. R., McGeorge, M., and Murphy, G. E.: The Action of Choline Esters in Myasthenia Gravis, *Clin. Sec.* 3: 77, 1937.
14. Ingvarsson, G.: Studies on Acetylcholine Sensitivity and the Velocity of Acetylcholine Splitting by the Human Blood, *Biochem. Ztschr.* 281: 370, 1935.
15. Galehr, O., and Plattner, F.: Fate of Acetylcholine in the Blood, *Arch. f. d. ges. Physiol.* 218: 488, 1928.
16. Hall, G. E., and Ettinger, G. H.: The Effect of Regular Injections of Acetylcholine Upon the Choline-Esterase Activity of Serum, *J. Pharmacol. & Exper. Therap.* 59: 29, 1937.
17. Stedman, E., and Russell, W. R.: The Cholinesterase Content of Blood in Myasthenia Gravis, *Biochem. J.* 31: 1987, 1937.
18. Fisher, R. A.: *Statistical Methods for Research Workers*, ed. 4, London, 1932, Oliver and Boyd, p. 114.
19. Hicks, C. S., and MacKay, M. E.: Cholinesterase Activity of Serum in Two Cases of Myasthenia Gravis, *Australian J. Exper. Biol. & M. Sc.* 14: 275, 1936; The Cholinesterase Content of Blood Serums From Normal and Myopathic Subjects, *Ibid.* 16: 39, 1938.
20. Easson, L. H., and Stedman, E.: Absolute Activity of Cholinesterase, *Proc. Roy. Soc., London, s.B.* 121: 142, 1936.
21. Stedman, E., and Stedman, E.: The Purification of Choline Esterase, *Biochem. J.* 29: 2563, 1935.
22. Matthes, M.: The Action of Blood on Acetylcholine, *J. Physiol.* 70: 338, 1930.
23. Corkill, A. B., and Ennor, A. H.: Choline Esterase in Myasthenia Gravis, *M. J. Australia* 2: 1121, 1937.
24. Russell, W. R., and Stedman, E.: Observations on Myotonia, *Lancet* 2: 742, 1936.
25. Antopol, W., Tuchman, L., and Schiffrin, A.: Choline-Esterase Activity of Human Serums With Special Reference to Hyperthyroidism, *Proc. Soc. Exper. Biol. & Med.* 36: 46, 1937.
26. Antopol, W., Schiffrin, A., and Tuchman, L.: Decreased Cholinesterase Activity of Serum in Jaundice and in Biliary Disease, *Proc. Soc. Exper. Biol. & Med.* 38: 363, 1938.
27. Sobotka, H., and Antopol, W.: Inhibitors of Choline Esterase, *Enzymologia* 4: 189, 1937.
28. Tod, H., and Jones, M. S.: A Study of the Cholinesterase Activity in Nervous and Mental Disorders, *Quart. J. Med.* 6: 1, 1937.
29. Jones, M. S., and Tod, H.: The Effect of Altering Conditions of the Autonomic Nervous System on the Cholinesterase Level in Human Blood Serum, *J. Ment. Sc.* 83: 202, 1937.
30. Altenburger, H.: Acetylcholine Breakdown in the Blood and Cerebro-Spinal Fluid, *Klin. Wchnschr.* 16: 398, 1937.
31. Hall, G. E., and Lucas, C. C.: Cholinesterase Activity of Normal and Pathological Serums, *J. Pharmacol. & Exper. Therap.* 59: 34, 1937.
32. Milhorat, A. T.: The Choline-Esterase Activity of the Blood Serum in Disease, *J. Clin. Investigation* 17: 649, 1938.
33. Long, C., and Lupton, H.: Fate of Lactic Acid After Exercise, *J. Physiol.* 67: 57, 1923.
34. Manning, G. W., Lang, J., and Hall, G. E.: The In Vivo Effects of Eserine on the Choline-Esterase System, *J. Pharmacol. & Exper. Therap.* 61: 350, 1937.
35. Ammon, R., and Voss, G.: The Acetylcholine-Destroying Action of Blood, *Arch. f. d. ges. Physiol.* 235: 393, 1935.
36. Dikshit, B. B., and Mahal, H. S.: Choline Esterase in Toxemia, *Quart. J. Exper. Physiol.* 27: 41, 1937.
37. Stedman, E., and Stedman, E.: The Relative Choline-Esterase Activities of the Serum and Corpuscles From the Blood of Certain Species, *Biochem. J.* 29: 2107, 1935.
38. Glick, D., and Antopol, W.: Inhibition of Cholinesterase by Thiamine (Vitamin B₁), *J. Pharmacol. & Exper. Therap.* 65: 389, 1939.
39. Minot, A. S.: A Comparison of the Actions of Prostigmine and Guanidine on the Activity of Choline Esterase in the Blood Serum, *J. Pharmacol. & Exper. Therap.* 66: 453, 1939.
40. Govaerts, P., Cambier, P., and van Dooren, F.: The Rate of Destruction of Acetylcholine in the Blood of Individuals Sensitive or Resistant to That Substance, *Compt. rend. Soc. de biol.* 108: 1178, 1931.

LABORATORY METHODS

GENERAL

IMPROVED INTUBATION TUBES FOR INSUFFLATION ANESTHESIA IN DOGS*

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SINCE pneumothorax results in bilateral lung collapse in the dog, intrathoracic surgery must be performed under some form of intratracheal insufflation. The simplest method consists of periodic inflation of the lungs by blowing air ether intratracheally. A close-fitting intubation tube¹ or the introduction of a tracheal cannula is, however, required.

Hamilton, Woodbury, and Vogt² (1939) advocate, for intrathoracic operations, an interrupted blast of air via a small tracheal catheter. This catheter carries a balloon, inflated through an opening in the catheter above its terminal one, which raises the pressure in the trachea during each blast. This method is said to be an improvement over other methods.

Livingstone and Hrdina³ (1930) developed a "modified Meltzer apparatus" which has been used successfully in their laboratory for many years. This simple apparatus delivers ether air at low pressures (compressed air passes through an ether "bottle") to the bifurcation of the dog's trachea via an urethral catheter (No. 16 French). Desired inflation of the lungs is accomplished by impeding the exhaust of gases through the trachea. This is accomplished by a modified Kunde mask into which the dog's muzzle is introduced through a perforation in a tight-fitting rubber dam. The other end of the mask (metal can) has two openings; one opening allows the passage of the insufflating catheter, the other is connected with a tube which leads the exhaust gases into an 8 liter water bottle. The catheter passes intrabuccally through a metal airway.

We have had occasion to use the above apparatus repeatedly in intrathoracic operations. The method has proved essentially satisfactory since one can easily maintain *apnea vagi* by moderate distention of the lungs. No untoward results of this anesthesia have been encountered, and the motionless thorax is a distinct advantage in these operations. We have found, however, disadvantages in the use of the Kunde mask, flexible catheter, and airway used by Livingstone and Hrdina.

Ether anesthesia for most operations in the dog is, we believe, given most satisfactorily by a tight-fitting metal intubation tube¹ which is introduced into the trachea through the larynx. A set of six twelve-inch tubes, ranging from $\frac{3}{4}$ to $\frac{7}{16}$ inches (outside diameter), will be satisfactory for dogs weighing less

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than 20 kg. Such tubes can be equipped with a beveled tip (to facilitate intubation) and nickel or chrome plated. Such a tube connected with an ordinary ether "bottle" by a short rubber tube eliminates the bulky Kunde mask and has proved entirely satisfactory. Atropine, preoperatively, is unnecessary.

Satisfactory anesthesia with the metal tubes led us to devise intubation tubes which are used for intratracheal insufflation anesthesia and replace the Kunde mask, metal airway, and long catheter used by Livingstone and Hrdina. This not only eliminates bulky apparatus but makes intubation much simpler.

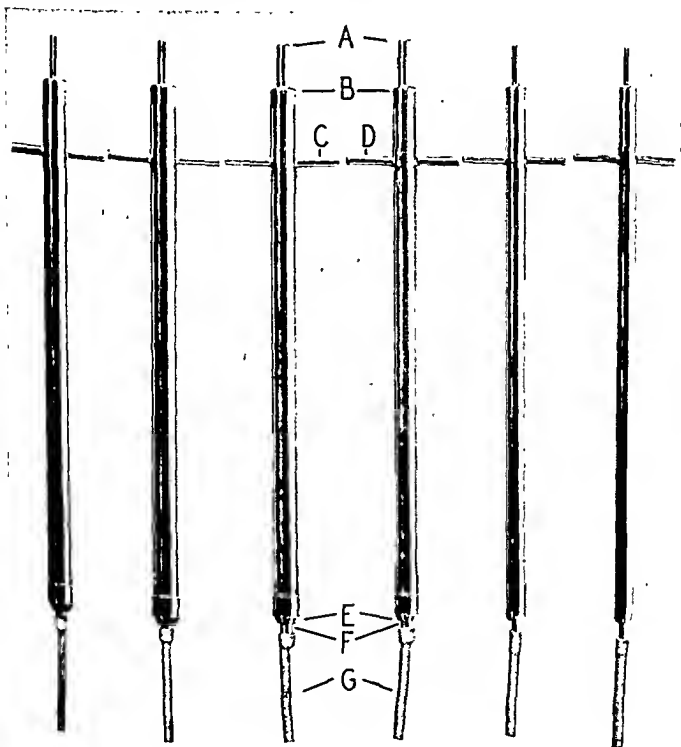


Fig. 1.

APPARATUS

The intubation tubes (Fig. 1) consist of six nickel-plated brass tubes, 12 inches long (standard seamless brass tubing of outside diameters: $\frac{3}{4}$, $\frac{11}{16}$, $\frac{5}{8}$, $\frac{9}{16}$, $\frac{1}{2}$, $\frac{7}{16}$ inches). A smaller tube ($\frac{3}{16}$ to $\frac{5}{16}$ inches, outside diameter), $13\frac{1}{2}$ inches long (A-F), is fixed inside the larger tube with A (upper end) protruding

1 inch while *F* (lower end) extends $\frac{3}{4}$ inch below the beveled tip *E*. The outer tube is closed completely about the smaller tube at *B*. Side tube *C* is closed but *D* communicates only with the space between the long inner and outer tubes. The lower end of the outer tube is fitted with a beveled tip *E* (see Fig. 2) which leaves an opening between the tubes. A 3 inch rubber tube *G* is attached to the inside tube *F*.



Fig. 2.

METHOD

One hour after administration of morphine sulfate (1 mg. per kilogram) the dog is anesthetized with ether. Under deep anesthesia the lower end of the intubation tube is introduced through the larynx on *inspiration* (the tube selected should fit the trachea snugly). The intubation tube is passed caudad so that the tip of rubber tube *G* lies just above the bifurcation of the trachea. Tube *A* is connected with the compressed air-ether source, and *D* is connected with the exhaust tube leading to the water bottle. The dog's jaws are bound tightly about the intubation tube, the bandage being carried around side tubes *C* and *D* to prevent slipping.

By this device an air-ether mixture can be blown down tube *A* under 2 to 4 cm. Hg pressure and carried to 1 cm. above the bifurcation of the trachea by tube *G*. Exhaust gases pass between the inner and outer tubes and out through *D*. The latter is connected with a metal tube which can be submerged (thus regulating the exhaust pressure) to the desired level. Pulmonary inflation to secure *apnea vagi* can be obtained easily by raising the exhaust pressure. This manipulation allows one to abolish all thoracic movement. Restoration of "negative" intrathoracic pressure is accomplished by lung distention at time of closure of the thoracotomy.

SUMMARY

An intubation tube for use with positive pressure apparatus for anesthesia in dogs undergoing intrathoracic surgery, together with its method of use, is described.

REFERENCES

1. Markowitz, J.: Textbook of Experimental Surgery, Baltimore, 1937, William Wood and Co., p. 23.
2. Hamilton, W. F., Woodbury, R. A., and Vogt, E.: Differential Pressures in the Lesser Circulation of the Unanesthetized Dog, *Am. J. Physiol.* 125: 130, 1939.
3. Livingstone, H., and Hrdina, L. S.: A Modified Meltzer Apparatus for Anesthesia in Animals, *J. LAB. & CLIN. MED.* 16: 74, 1930.

A SIMPLE AND COMPACT ERGOGRAPII FOR STUDENT USE*

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THE instrument to be described has several advantages over ergographs in common use at present. It is readily stored, having over-all dimensions of 18 by 6½ by 4 inches. It permits recording of excursions on drum. It records on an automatic counter the total distance through which force is applied. It can be used with unlimited stroke, or the length of stroke can be fixed so that uniform strokes are made. Its principal disadvantage is that experiments demonstrating the effect of varying the load on muscular ability can be done only if several machines of different inherent resistance are available for the student to use. The considerable variety of experiments which can be performed with fixed load make the machine useful despite this disadvantage.

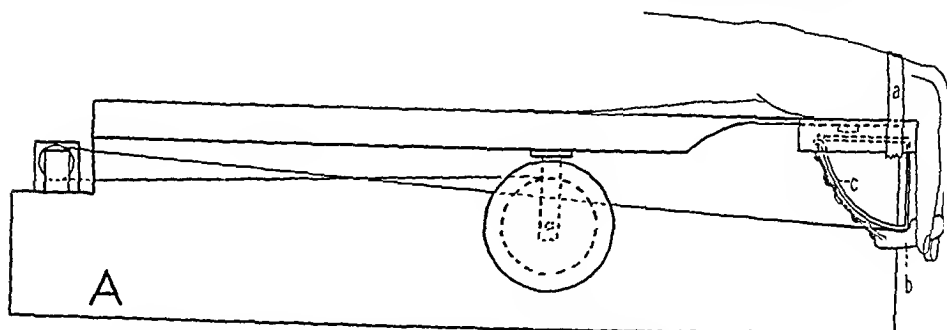
The Ergograph.—Fig. 1A shows a simple sketch of the machine with fingers in place ready for use. Note that the muscle used is the *extensor digitorum communis*. A consideration of the anatomy of the forearm and hand will show that with the metacarpus firmly fixed to the board by strap (*a* in Fig. 1A) no other muscle can apply force to the work.

The fingers are attached to the instrument by means of a rawhide thong. Fig. 1A shows the instrument seen from the side (sideboard removed) with the fingers in place in the thong (*b*). Fore, middle, and ring fingers are inserted. Connection is made from the thong by means of wire to the furthest point of a metal quarter circle (*c* in Fig. 1A and B). This piece is made of sheet brass 0.036 inch thick, 2 inches wide, and 4 inches long, bent to a right angle (Fig. 2A) to which is soldered a piece of the same sheet brass curved to a radius of curvature 2 inches. The result seen from the side is a quarter circle (Fig. 2B). The wire is attached at the uppermost end of the curved sheet. The center of the quarter circle is now soldered to a brass rod 6 inches long and 3/16 inch in diameter at about its center (Fig. 2C). This forms the axis to which force is applied. The ends of this rod are drilled out to receive a piece of hardened steel rod 1/8 inch in diameter which is allowed to project from the end of the rod to act as a bearing (Fig. 2D). These bear on holes of proper size drilled in side pieces of brass rectangular bar 1/8 by 3/4 inch which are recessed and screwed to the sides of the top board of the ergograph. The load factor or resistance to the movement of the axle is supplied by a coiled music wire spring whose lumen diameter is about 1/4 inch.† It is slipped over the axle and anchored to the brass quarter circle on one end, and to the bearing plate on the other (*d* in Fig. 1B). Friction between the axle and the spring is prevented by having the lumen diameter of the spring greater than the diameter of the axle and also by soldering a small brass collar (*a* in Fig. 2E) to the bearing plate within which the axle fits and on the outside of which the spring rides. The resistance

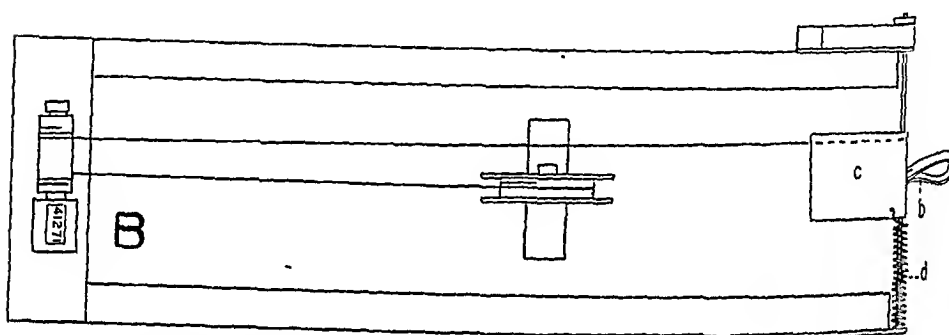
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†Music wire 0.048 inch in diameter fashioned into a spring of 1/4 inch inside diameter having 23 turns in 2 inches length tightened by one full turn before being anchored produces about 100 Gm. tension, which is a good resistance for untrained *extensor digitorum communis* muscles.

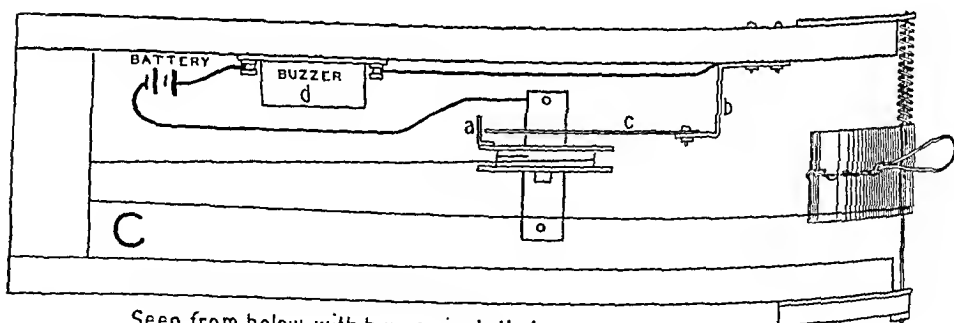
to turning of the axle will depend on the strength of the spring wire used and on the number of turns made on it before it is anchored. The finger thong is attached around the circle so that the force will always be applied at a fixed distance from the axis.



Seen from side, as if side board were transparent



Seen from above, as if top board were transparent



Seen from below with buzzer installed

Fig. 1.

The same principle is used in activating the meter to count the number of centimeters through which the force is applied. A small hole is drilled in the anterior end of the curved brass plate and a cord is fixed in this hole (Fig. 2F). The cord runs backward under the arm board to a spindle to which it is anchored after several turns. The spindle is 5 cm. in circumference and 1.5 inches long. It is held up on one side by a brass bearing and on the other by the shaft of a Veeder rotary ratchet counter (type Q13) to which the spindle is fixed. Each rotation of the spindle will turn the counter through 10 units and will imply that force has been applied through 5 cm. Thus each unit on the counter is 0.5 cm. Since turning the axle pulls the cord over an arc at a constant distance from the axis of rotation of the axle, the distance indicated by the counter will be correct.

After a movement the axle will be returned to normal position by the spring. The cord must be returned by a separate mechanism. This is accomplished by applying a second cord to the spindle which after several turns passes forward under the arm board to a spring wheel (Fig. 2G) which exerts a tension tending to unwind the cord from the spindle. Thus when the axle is turned, the cord is wound off the left side of the spindle, activating the counter. Simultaneously, the cord is also wound on the right side of the spindle from the spring wheel. After the force is released, the spring returns the axle to place, freeing the axle cord to the spindle. The tension of the spring wheel brings the cord back from the spindle onto the wheel and thus returns the axle cord to the spindle. The counter is prevented from backward rotation by its inherent ratchet.

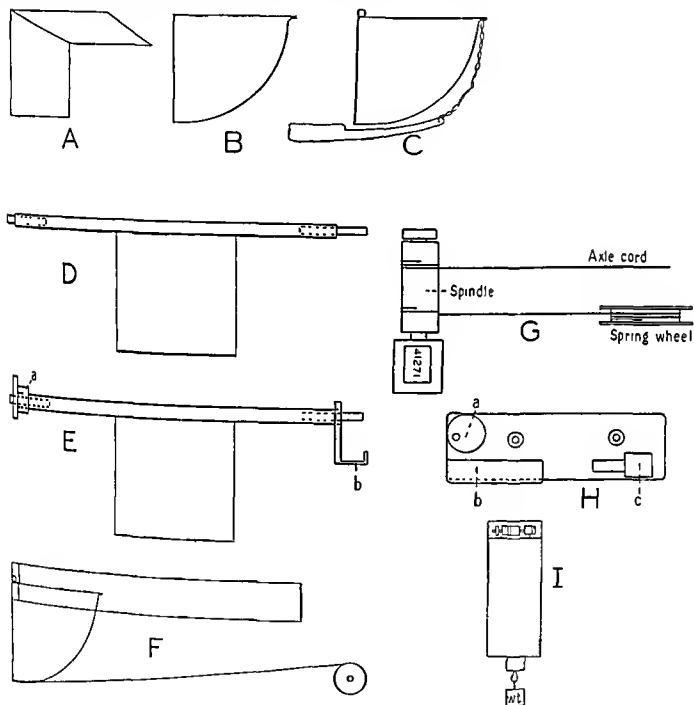


Fig. 2.

The ergograph is designed to record on drum through air transmission to a tambour with fall-away lever. The hardened steel bearing peg inserted in the shaft on the side opposite the spring is made long enough to extend $\frac{1}{2}$ inch beyond the bearing plate (see Fig. 2D and E). To this peg is fixed an eccentric cog $\frac{1}{2}$ inch in diameter and $\frac{1}{4}$ inch thick (a in Fig. 2H). Below this cog, and fixed to the bearing plate, is a brass trough (b in Fig. 2E and H) which lies $\frac{1}{8}$ inch below the base of the eccentric cog when the axle is twisted to its maximum stroke. The trough is $\frac{5}{16}$ inch wide. A rubber tube is inserted between the cog and trough. At the posterior end of the trough there is soldered to the brass plate a brass rod projection (c in Fig. 2H) upon which the rubber tubing can be

fixed so that it is firmly held and prevented from leaking air. The other end of the rubber tube is attached to the tambour. In actual use the strokes upon the drum can be readily translated into centimeters of excursion by calibrating the extent of excursion per given distance as measured on the counter.

For certain applications it is desirable to make the ergograph strokes uniform. This is readily accomplished by arranging contacts so that a buzzer sounds when the stroke reaches a given distance. Fig. 1C shows contacts and buzzer installed. A brass rod $\frac{1}{2}$ by $\frac{1}{4}$ by $\frac{1}{8}$ inch is soldered to the outer edge of one flange of the spring wheel so that it projects radially (*a* in Fig. 1C). To the undersurface of the arm board there is affixed a metal bar (*b*) which acts as a base for a swing lever (*c*) of brass 0.025 by 0.125 by 4.0 inch. The swing lever is prevented from downward swing by crimping its end behind the fulcrum, and prevented from upward swing by a simple spring wire attached to the fulcrum. Thus it is held horizontal $\frac{1}{2}$ inch below the undersurface of the sideboard. An electric door buzzer (*d*) is fixed to one of the sideboards. A dry cell is connected to one pole of the buzzer and to the base of the spring wheel. The second buzzer pole is then connected to the swing lever holder.

When the fingers are extended, the spring wheel is rotated until the projecting contact strikes the swing lever. The circuit is then completed, ringing the buzzer. The subject pulls until the buzzer sounds, relaxes, and pulls again, making uniform strokes. If the buzzer is disconnected, or if stroke is continued despite the buzzing, nothing prevents the wheel from rotating as the spring of the swing lever readily gives. Thus the buzzer can be left out of operation by simply disconnecting the battery.

The advantage of stroke uniformity is that it establishes a neat end point to call fatigue. Fatigue can be defined as that condition of the person in which he cannot make a stroke of this length in the given time interval.

Some Applications of the Ergograph for Student Use.—

1. Demonstration of *treppe*—human fatigue curves.
2. Comparison of work a muscle can do circulated with the work it can do when ischemic.
3. Effect of rate of contractions on total work ability of muscle, (a) circulated or (b) ischemic.
4. Effect of motivation on muscular ability—comparison of work output ringing buzzer with work output without buzzer.
5. Effect of massage on muscular ability.
6. Effect of fatigue of other muscles on the work of an unused muscle.
7. Effect of ischemic work on the blood pressure and heart rate (reflex of Alam and Smirk).
8. The character and properties of ischemic pain.

(All these experiments except No. 4 can be done with or without the buzzer.)

Calculation of the Work Done on the Ergograph.—The resistance against which the force works is that of a spring. This means that the resistance increases gradually throughout the stroke. The resistance can be calibrated by hanging weights on the finger thong with the ergograph suspended in a vertical position (as in Fig. 2I). Gravity acting on the weights will overcome the resistance of the spring. If gravity acting on a weight of 1,100 Gm. moved the counter 4 units (2.0 cm.), then $1,100 \times 2.0 = 2,200$ gram centimeters of work

must be expended every time a stroke 2.0 cm. long is made. On a particular machine the following calibration was obtained:

Weight Suspended	Average Movement Recorded
1,100 Gm.	1.3 cm.
1,150 Gm.	2.8 cm.
1,200 Gm.	3.5 cm.
1,250 Gm.	4.5 cm.
1,300 Gm.	6.8 cm.
1,350 Gm.	9.0 cm.

This implied that a stroke of 6.8 cm. required the same work as would have been required if a constant load of 1,300 Gm. had been used, and that $6.8 \times 1,300 = 8,840$ gram centimeters of work were required to make the stroke of this length. For student use a simple method of calculation was desired. With the buzzer in use there was no difficulty, since all strokes were uniform and gravitational calibration gave the uniform load equivalent of this stroke. Thus the work was calculated as the product of distance times this equivalent. When the buzzer was not being used and the length of the stroke varied widely, it was questionable whether a gravitational equivalent based on average stroke length would be sufficiently accurate. The average stroke length in any given work bout was readily obtained by dividing the distance recorded on the counter by the number of strokes made by the subject. The number of strokes can be counted as made or from the kymogram. Several methods of calculation suggested themselves and were checked on twenty different records made on various experimental procedures.

Method 1. From the twenty different trials the average stroke for all trials was 6.4 cm. Referring back to the calibration and interpolating, the uniform load equivalent was about 1,290 Gm. Multiplying the distance factor in each bout of work by 1,290 then approximated the work in gram centimeters.

Method 2. Another possibility was to use the average stroke for each particular bout of work. In the twenty different trials the average stroke varied from 5.1 to 7.8. The distance in the bout of 5.1 cm. average stroke was multiplied by 1,260 Gm. to obtain the work; the distance in the 7.8 bout was multiplied by 1,320 Gm. to obtain the work.

Validification of Method.—Both methods 1 and 2 arrive at approximations, and while it is obvious that the work results by the two methods can show no greater variation in these twenty records than 3 parts in 129, one has no idea how far the result by either method is from the true work done.

Method 3. The work done in each bout was determined accurately as follows: Before each of the twenty work bouts a calibration of centimeters of stroke against millimeters of tambour excursion was made. This is rendered easy by the automatic counter, since by watching the counter, strokes of 1 cm., 2 cm., 3 cm., etc., can be made. After the record was fixed, each stroke was translated from millimeters to centimeters so that an incidence record resulted. Table I shows the incidence of different strokes in a particular record. It can be seen that the strokes varied from 2.25 to 8.0 cm. in length. Referring back to gravitational calibration, the gravity equivalent of each one of these strokes was obtained as seen in the fourth column of Table I. Now the centimeter equivalent was multiplied by the incidence of such strokes, and the product was

multiplied by the gravity equivalent of this length of stroke. The sum of these results gave the work done. The work calculated by methods 1 and 2 in this case was $498 \times 1,290 = 643,000$ (1) and $498 \times 1,295 = 646,000$ (2).

TABLE I
CALCULATION OF WORK BY INDIVIDUAL STROKES
(Incidence From Tambour Record)

MM.	CENTIMETER EQUIVALENT		INCIDENCE		GRAVITATIONAL EQUIVALENT		
11	8.0	x	2	x	1,325	=	21,200
10	7.4	x	21	x	1,310	=	204,000
9	6.75	x	34	x	1,300	=	298,000
8	6.25	x	9	x	1,290	=	72,500
7	5.75	x	1	x	1,275	=	7,300
6	5.10	x	1	x	1,260	=	6,400
5	4.5	x	3	x	1,250	=	16,900
4	4.0	x	2	x	1,225	=	9,800
3	3.4	x	2	x	1,200	=	8,200
2	2.25	x	2	x	1,125	=	5,100
							649,400

One may wonder whether the ability to measure tambour strokes on a record is good enough to warrant calling method 3 accurate. There is a ready check on the accuracy of the measurement as follows: If in Table I the centimeter equivalents are multiplied by the incidence alone and all the products are summed, the result is the total distance through which the force was applied; in this case the sum was 500.9 cm. But the counter also added up the total distance during the work bout as 498 cm. Thus the measurement is checked.

This procedure was carried out with each of the twenty records, and the percentage error of work as calculated by methods 1 and 2 from method 3 taken as accurate varied from -3.9 per cent to +2.1 per cent for method 1; from -3.1 per cent to +1.7 per cent for method 2. We conclude that the error involved by either method 1 or 2 is small; that method 2 is somewhat less subject to error than method 1.

For student use we furnish a weight calibration with each instrument and instruct the student to use method 2. After the instrument is assembled it requires a week or two for the spring to take its set. The force of the spring seems to remain quite constant once the set is taken. Probably gravitational calibration at least once a year will be necessary.

SUMMARY

A student ergograph for human extensor digitorum communis has been described. Its over-all dimensions are 18 by $6\frac{1}{2}$ by 4 inches. It is economical to construct. It permits limitation of stroke length so that fatigue can be more sharply defined as an end point. A list of experiments to which it can readily be applied is given. Methods available for calculation of the work done on the machine are discussed and validated. The portability of the ergograph recommends it for clinical use in following the work ability of patients under treatment for Addison's disease, and myasthenia of any other origin in which the various muscles are fairly uniformly affected.

THE USE OF A SINGLE GUINEA PIG FOR VIRULENCE TESTING*

MARGARET BEATTIE, M.A., GR.P.H., BERKELEY, CALIF.

IN A PREVIOUS study¹ it was demonstrated that the use of a single rabbit for virulence testing by means of the coincident intracutaneous injection of a test and a control suspension of *C. diphtheria* culture mixed with antitoxin (instead of the parallel injection of the culture suspension into an unprotected and antitoxin protected animal) so enhanced the inherent variability of rabbit reactivity to diphtheria toxin as to yield falsely negative reactions in tests with known pure cultures of *C. diphtheriae*. This modification of the traditional technique was introduced by Fraser, Halpern, and Roy² as an economical way to test the large number of cultures involved in research or survey investigations. Only under extraordinary circumstances would a public health laboratory have so many tests to perform at one time or have rabbits available for this purpose. In such a laboratory the economy involved in the use of a single animal for a few tests would be desirable. In health department laboratories it is important to discover immediately whether organisms, with morphology characteristic of *C. diphtheriae*, when isolated from the throats of carriers, recovered patients, or immunized contacts, are virulent. The infectiousness of the immunized child whose throat lesion yields organisms morphologically resembling diphtheria bacilli is also important to health authorities.

At the time the experiments on rabbits were done, a few guinea pigs were tested by this technique. These tests indicated that while the guinea pig was less apt to vary in reactivity to the injections than the rabbit, some differences in reaction occurred in animals injected with control suspensions. In order to limit the effect of antitoxin, the number of tests to be placed on each animal was reduced from eight to four. The amount of antitoxin in the control suspension was decreased to what appeared to be the least amount for neutralization of toxin.

Cultures.—Four cultures were selected from the sixteen which had been used on rabbits. One (No. 75), a gravis strain, was chosen as a control culture because it had reacted positively on all rabbits and guinea pigs. Two intermediate strains (Nos. 133 and 198) which had frequently yielded inconclusive or negative reactions were included as test strains. The fourth test culture was a mitis strain (No. 206) which had occasionally induced negative or inconclusive reactions on rabbits.

Test Inoculum.—In order to obtain a sufficient amount of culture suspension, several slopes of Loeffler's medium were inoculated with each test culture. This was accomplished by delivering with a pipette a saline suspension made

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from the growth on a Loeffler's slant onto the surface of each of several slopes. Complete seeding of the surface was accomplished by rotating the inoculum over the surface of the slopes before placing the tubes in the incubator. After forty-eight hours' incubation the growth on each slope was suspended in 2 c.c. of Stone's suspending solution.³ A stained smear from each test culture was examined to check the presence of morphologically typical bacilli in pure culture. Subcultures were made from one tube of each set of cultures to be certain the material was viable. Because of the color and consistency of the suspending solution, only a rough estimation of the relative opacity of the suspensions could be made.

Control Inoculum.—To each cubic centimeter of suspension prepared as described above, 0.1 c.c. of antitoxin, containing approximately 100 units, was added. The suspension was well mixed and allowed to stand at room temperature for at least half an hour before being injected. This amount of antitoxin was evidently near the minimum because very slight necrotic reactions were occasionally observed.

Animals.—Of 129 guinea pigs all were white females except eleven which were white males. All animals were fully mature and weighed more than 400 Gm. though they varied considerably in weight, size, age, and skin texture.

THE EXPERIMENT

Five groups of from twelve to forty animals were tested within a three months' period. In each experiment the sex and size of the animals were considered in selecting those to be compared as test and control animals. Test animals received intracutaneous injections of both test and control culture suspensions coincidentally. Control animals received injections of test culture suspensions only. Each animal received the same culture suspension in the same skin area for any experiment. One-tenth cubic centimeter of inoculum was injected intracutaneously into the shaven skin on the side of guinea pigs. The control animals received 1,000 units of antitoxin five hours after the tests were placed. Both test and control animals received 1,000 units of antitoxin twenty to twenty-six hours after the tests had been placed. The reactions were observed at twenty-four-hour intervals for three days. Reactions were designated as positive when an area of necrosis resulting in a scab more than 4 mm. in diameter was formed; inconclusive when a small scab (less than 4 mm.) was observed; and as negative when no area of necrosis was noted.

RESULTS AND DISCUSSION

One hundred and twenty-nine guinea pigs were injected. Sixty-five guinea pigs (test animals) were injected with both test and control culture suspensions. Sixty-four guinea pigs (control animals) received injections of test suspension alone. Only one culture (No. 198) induced inconclusive and negative reactions which indicated variation of reactivity in the guinea pigs tested.

Of the sixty-five test animals, 53 reacted positively to all injections. Five animals had inconclusive reactions and seven gave negative reactions at the site of the injection of one test culture (No. 198).

Sixty-three of the 64 control animals yielded positive reactions to all test injections. One animal, on which all reactions developed slowly and were small, showed an inconclusive reaction at the site of injection of one test culture suspension (No. 198).

Differences in time and rate of development and size of lesion were apparent in different animals, but, on the whole, the lesions on the test animals were obviously smaller, particularly the lesions induced by the culture (No. 198) which was the sensitive indicator of any variation in guinea pig reactivity. The diameter of the scabs was measured in one group of 40 animals in order to verify this apparent difference. Culture No. 198 on the control group of guinea pigs induced scabs whose average diameter measured only 0.2 mm. less than the smallest average scab measurement of the three other cultures tested, whereas in the test series the scabs induced by this culture had an average measurement 2.0 mm. less in diameter than the other cultures. These measurements completely confirmed the observation that the reactions were larger on the animals of the control series.

It is evident that the use of control inocula containing antitoxin modifies the reaction on single animals injected intracutaneously for diphtheria virulence testing so as to render erroneous results. It might be possible to limit the application of the technique to one or two tests by determining the maximum amount of antitoxin which could be safely used, but this would involve the use of antitoxin closely measured for maximum as well as for minimum unit content. Such antitoxin is not available in the public health laboratory. A single animal can be utilized by repeating the injection of the test suspension in another skin area of the animal converted into a control by the intravenous injection of antitoxin five hours after the test injections are placed. This modification of technique was described by Fraser and Weld in 1926.⁴ The time interval and the labor involved in repetition of the injections frequently complicates the schedule so as to render the test inconvenient in a public health laboratory.

CONCLUSIONS

Guinea pigs vary only slightly and much less than do rabbits in their reactivity to the injection of cultures of *C. diphtheriae*. The coincident intracutaneous injection of a control mixture of culture and antitoxin which permits the use of a single guinea pig for several tests cannot be recommended since false negative or inconclusive reactions may occur.

REFERENCES

1. Beattie, Margaret: The Use of a Single Animal for Testing the Virulence of *C. diphtheriae*, J. LAB. & CLIN. MED. 25: 1111, 1939.
2. Fraser, D. T., Halpern, K., and Roy, I. S.: Studies in Diphtheria, Canad. Pub. Health J. 29: 40, 1938.
3. Stone, R. V., and Weigel, C.: A New Suspending Medium Used for the Intracutaneous Virulence Test of *Bacillus diphtheriae*, Am. J. Pub. Health 9: 113, 1929.
4. Fraser, D. T., and Weld, C. B.: The Intracutaneous Virulence Test for *Corynebacterium diphtheriae*, Tr. Roy. Soc. Canada 20 (sec. V): 343, 1926.

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REFERENCES

1. Beattie, Margaret: The Use of a Single Animal for Testing the Virulence of *C. diphtheriae*, J. LAB. & CLIN. MED. 25: 1111, 1939.
2. Fraser, D. T., Halpern, K., and Roy, I. S.: Studies in Diphtheria, Canad. Pub. Health J. 29: 40, 1938.
3. Stope, R. V., and Weigel, C.: A New Suspending Medium Used for the Intracutaneous Virulence Test of *Bacillus diphtheriae*, Am. J. Pub. Health 9: 113, 1929.
4. Fraser, D. T., and Weld, C. B.: The Intracutaneous Virulence Test for *Corynebacterium diphtheriae*, Tr. Roy. Soc. Canada 20 (sec. V): 343, 1926.

ON THE PRESERVATION OF SPECIFIC ANTI-M AND ANTI-N AGGLUTININS WITH TOLUOL*

PHILIP LEVINE, M.A., M.D., NEWARK, N. J.

IT IS well known that antisera, when preserved in the cold in the filtered state, retain their antibody content almost undiminished over a period of several years. For the production of diagnostic anti-M and anti-N reagents, it is necessary to remove the species-specific agglutinins by absorption with suitable bloods lacking the factors in question. For this purpose, the serum in a dilution of 1:15, 1:20, or higher is absorbed for one-half to one hour with one-half its volume of washed blood sediment. Generally, one absorption suffices to remove the species-specific agglutinins, but occasionally a second treatment, with perhaps a smaller quantity of blood, may be required. While these manipulations can be carried out under sterile conditions, this is not convenient, and consequently the diagnostic fluids become unsuitable for use after a few days in the ice chest because of bacterial contamination. Once potent diagnostic reagents are prepared, it is of considerable advantage to preserve them for future use on account of the large quantity of selected blood necessary for the absorption and the time and effort required for the numerous manipulations.

A number of years ago I¹ tested a few preservatives and observed that the addition of 1 drop of toluol per cubic centimeter of diagnostic fluid, stoppered and sealed with paraffin, gave very good results. Tests with diagnostic fluids preserved in this manner for a period of six to twelve months have invariably given unequivocal results, so that the tests always gave a sharp distinction between bloods possessing and bloods lacking the agglutinogens in question. Those rare bloods reacting weakly with potent diagnostic fluids, freshly prepared, were invariably agglutinated by the preserved fluids. At no time in an active, continued experience, extending over a period of ten years with the use of fluids preserved in this manner, were any difficulties encountered in the diagnosis of the M and N factors.

In a study of various preservatives of diagnostic fluids, Boyd² comes to the conclusion that there are a number of preservatives (such as brilliant green or acriflavine) which are preferable to toluol. However, Boyd made no tests with toluol preserved fluids before four and one-half years elapsed, at which time all activity had disappeared.

For this reason, I made tests with anti-M and anti-N diagnostic fluids preserved with toluol for a period of thirty-two months. These were the only fluids available to test the efficacy of the preservative, but it seems to me that a preserved diagnostic fluid still potent after an interval of thirty-two months in the ice chest is certainly properly preserved.

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Actually, these specific diagnostic reagents were found to give potent reactions that compared favorably with those obtained in simultaneous tests with freshly prepared reagents from the same antisera (Tables I, II, III).

TABLE I

DIAGNOSTIC FLUID	GROUP	O	A	A	A	A	A	B	O	A	A
	TYPE	M	M	M	MN	MN	MN	MN	N	N	N
Anti-M (1)		++++	++++	++++	++++	++++	++++	++++	0	0	0
Anti-M (2)		++++	++++	++++	++++	++++	++++	++++	0	0	0
Anti-N (3)		0	0	0	±	±	±	±	++++	++++	++++
Anti-N (4)		0	0	0	++	++	++	++	++++	++++	++++

The plus signs indicate varying degrees of agglutination.

(1) Prepared from serum 136, diluted 1:15, and preserved with toluol on July 3, 1937.

(2) Freshly prepared from serum 136, diluted 1:15.

(3) Prepared from serum 52, diluted 1:20, and preserved with toluol on July 3, 1937.

(4) Freshly prepared from serum 52, diluted 1:20.

TABLE II

TITRATION OF THE ANTI-M FLUIDS						
		1:6	1:12	1:24	1:48	1:96
Anti-M No. 136 preserved for 32 months	(1) OM	++++	±	+	tr	0
	(2) AM	++++	+++	+	0	0
	(3) OMN	++++	+++	+	0	0
	(4) AMN	++++	±	±	0	0
Anti-M No. 136 fresh	(1) OM	++++	±	+	0	0
	(2) AM	++±	+	0	0	0
	(3) OMN	++±	±	tr	0	0
	(4) AMN	±	+	0	0	0

tr. indicates trace.

TABLE III

TITRATION OF THE ANTI-N FLUIDS					
		1:6	1:12	1:24	1:48
Anti-N No. 52 preserved for 32 months	(1) ON	±	+	tr	0
	(2) AN	±	+	f.tr.	0
	(3) OMN	±	0	0	0
	(4) AMN	+	±	0	0
Anti-N No. 52 fresh	(1) ON	++±	+	f.tr.	0
	(2) AN	±	+	tr	0
	(3) OMN	+	tr	0	0
	(4) AMN	±	+	0	0

tr. indicates trace; f.tr., faint trace.

It is clear that the anti-M diagnostic fluids, preserved with toluol for thirty-two months, is just as active as the fluid freshly prepared from the same serum; the preserved anti-N fluid gave reactions which were only slightly less intense than those with the freshly prepared fluid. Titrations of the fresh and preserved anti-M fluid gave results which indicate that the preserved fluid is, indeed, somewhat stronger than the freshly prepared reagent. Similar slight differences of behavior of the two diagnostic fluids were observed in testing with other bloods, not recorded in Table II. The titration of the anti-N fluids gave results which confirm those of Table I, namely, a slightly greater activity on the part of the freshly prepared fluid.

Obviously, there are other suitable chemical preservatives for specific anti-M and anti-N reagents, as has been shown by Boyd. Another suitable procedure would seem to be drying with some such technique as the Flosdorf-Mudd apparatus. In any event, the results here reported indicate that toluol may be just as efficient a preservative as, for instance, aeriflavine, especially since Boyd's anti-M fluid No. 887, preserved for twenty-two months with aeriflavine, already showed a considerable diminution in activity.

Addendum: Retest of the same diagnostic fluids on January 20, 1941, i.e., after preservation of three and one-half years, showed no loss of activity in the anti-M reagent. The anti-N reagent suffered a drop of titer, distinct reactions obtainable at a dilution of 1:6 instead of 1:12; tested in the undiluted state, the anti-N diagnostic fluid gave clear-cut differentiation.

REFERENCES

1. Levine, Philip: Unpublished experiments.
2. Boyd, W. C.: Production and Preservation of Specific Anti-Sera for Blood-Group Factors A, B, M, and N, *J. Immunol.* 37: 65, 1939.

A SIMPLE, INEXPENSIVE, VERTICAL ILLUMINATOR FOR THE STUDENT MICROSCOPE*

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THE need for vertical illumination for examining human skin capillaries and minute vessels in the frog in the student laboratory directed attention to the possibility of using for this purpose the very effective pencil flashlight bulb known as Mazda 222 (operating on 2.2 volts). A simple, flexible, convenient, and effective mounting was provided with a dial socket, screw type (costing four cents), carried by a copper wire, 10 inches in length and $\frac{1}{8}$ or $\frac{1}{4}$ inch in diameter. This in turn was clamped to the ring stand. The beam of light could then be directed to the vascular area to be observed. The arrangement can be brought close enough to provide enough light for observation of the minute vessels unless high magnifications are required. When strong illumination is desired for microphotography, the bulb may be operated momentarily above its rated voltage by inserting a single pole double throw toggle switch in the lead to the bulb and providing a source of slightly higher voltage.

The current source may be dry cells, but in the student laboratory we operate a group of bulbs on one filament transformer supplying a.c. at 2.5 volts. A short piece of resistance wire in the lead to the bulb reduces the voltage to the correct value. When the bulbs are operated below their rated voltage, they last twenty-four hours or more in continuous operation.

We placed thirty of these illuminators in the student laboratory at a cost of less than ten dollars. They proved very satisfactory for student study of the minute vessels. Other uses will suggest themselves.

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THE USE OF DIAPHANE FOR MOUNTING GIEMSA TYPE PREPARATIONS*

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IN LABORATORIES where Romanowsky-type stains are used, it has always been a problem to find a satisfactory as well as permanent mounting medium. This is especially true of those laboratories where malaria research is done. For here the routine method of staining the parasites invariably consists of the use of one of the better Romanowsky stains, such as Giemsa's, MacNeal's tetrachrome, or Wright's.

In general, three types of preparations are used; namely, blood smears, and smears and sections prepared by dehydration. When these are mounted in balsam, the stain almost always fades considerably within a few months or a year. This fading is usually found in the region of the smear or sections farthest from the edge of the cover slip, leaving a rim of fairly well-stained material. The reason advanced for this gradual destaining process is that the α and β abietinolic acid in the balsam gradually oxidizes, thus changing the pH of the medium. And since the Romanowsky stains work best in an alkaline state, this causes them to fade.

Many alterations in the balsam have been suggested to counteract this tendency toward acidity. In Lee's¹ *Vade Mecum* (1928, p. 231) a method of making neutral balsam is given which consists essentially of mixing the balsam crystals with an equal amount of sodium bicarbonate. Scott² (1911) recommends placing a piece of marble in the balsam bottle and protecting the bottle from light. A similar suggestion is made by Guyer³ (1936, p. 129). This works satisfactorily for awhile but fading eventually occurs. Cannon and Talinferro⁴ (1931) mounted sections in balsam without cover slips, stating that this prevented rapid decolorization. Huff and Bloom⁵ (1935) used a petrol-ether soluble fraction of crude dammar dissolved in xylene as a mounting medium. Kingsley⁶ (1937) recommends keeping stained megakaryocytes and platelets in sections mounted in neutral xylene dammar away from light. Lee (p. 503) suggests mounting Giemsa-stained sections in cedar oil rather than in balsam, since in the latter the colors fade more rapidly.

In 1927 Wright⁷ showed that euparal was an excellent mounting medium for blood smears stained with the many Romanowsky-type stains. Slides so mounted did not fade for over six years. Euparal was introduced by Gilson⁸ (1906) who stated it was a mixture of eucalyptol, paraldehyde, and eamsal. A similar American product is sold by some supply houses under the name of diaphane. This product is said to contain euparal, ether, gummed sandarac, and paraldehyde. Shepherd⁹ (1918) presented the formula of a substitute for euparal which he claimed was in all respects similar to it. Both euparal and diaphane have a refractive index of 1.483. Each is available in a colorless and a green form. The latter contains a copper salt, the color of which is said to

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intensify hematoxylin stains. The colorless diaphane is the proper one to use for mounting Romanowsky-type stained preparations. Histologic comparisons of diaphane and euparal indicate that they are identical in properties. Therefore, in this paper when reference is made to diaphane, it is implied that the same is true of euparal.

Diaphane is superior to balsam in many respects, and especially in that it is a nonoxidant and is neutral to all stains. It also has a lower index of refraction, enabling observation of objects totally invisible in balsam or dammar. Most materials may be mounted in it directly from 95 per cent alcohol.

Recently, Groat¹⁰ (1939) described two new cycloparaffin mounting media, commercially called Nevillite V and No. 1. These substances are similar to balsam and are soluble in toluene and xylene. They are said to be superior to balsam in that they do not become acid with age. However, they have higher refractive indices (Nevillite V, 1.544; Nevillite No. 1, 1.567) than balsam (1.535), which makes them optically much inferior to diaphane (1.483). Furthermore, Nevillite must be used with absolute alcohol and a clearing agent such as xylene.

MATERIALS AND METHODS

Two simple and efficient techniques were developed permitting the use of diaphane as a mounting medium after staining with Giemsa's, Wright's, Jenner's, and the Eosin Azure II method of Maximow's. In general, the following discussion applies specifically to preparations stained with Giemsa's, since it is used routinely in this laboratory.

The first technique is to be used with alcohol-acetone mixtures, and the second with dioxane as the dehydrating agent. Both give approximately the same results. The advantage of the one method over the other depends on the circumstances in which they are to be used. For ordinary work, or when it is difficult to obtain dioxane, the alcohol method will be most convenient and inexpensive. Since absolute alcohol is not necessary, it will be found especially valuable for work in humid climates, such as the tropics. The dioxane method gives advantages familiar to all who have had occasion to use dioxane.

The staining technique employed was essentially a simplification of Wolbach's¹¹ (1919) method for staining paraffin sections with Giemsa. We have used the technique in staining smears and tissue preparations of bird malaria, Hemoproteus, and fowl pox. Malaria and Hemoproteus parasites stain blue in contrast to the red blood cells which stain a light yellow orange. The general cellular substance takes a rich blue appearance. In fowl pox, the Borrel and Bollinger bodies stain bright red against a blue background. A good account is given in Lee (p. 503) with many helpful suggestions. Hewitt¹² (1939) devised a modification of the Wolbach method which gives fine results for malaria parasites. However, this method is time-consuming and the dehydration and clearing procedure may be much abbreviated when diaphane is used instead of the cedar oil or neutral balsam recommended.

STAINING PROCEDURE

A. *Dry blood smears* were made in the usual manner by running a small drop of blood on the end of one slide along another. The smears were air-dried

and then fixed in absolute methyl alcohol (reagent). They were then allowed to dry and stained with a mixture of 2 drops of the stock Giemsa to each cubic centimeter of water used. After ten to thirty minutes they were removed, washed, and dried. If the slides are to be mounted, diaphane may be applied directly to the dry smear covering with a No. 1 cover slip.

B. *Smears and tissues prepared by dehydration* were always fixed in Maximow's Zenker-formol (10 per cent formalin; no acetic acid). Preparations were immersed in this fluid immediately to avoid drying. Blood or tissue smears were fixed for thirty minutes or less; small pieces of tissue for two to three hours.

(1) *Smears* were washed in water (running preferred) for at least five minutes. If *alcohols* are to be used, pass through to 70 per cent alcohol slightly colored with iodine. Leave for ten to fifteen minutes and remove. Pass smear back through the alcohols to water. Mordant with a 2.5 per cent solution of potassium bichromate for five to ten minutes. Wash and stain with a mixture of 4 drops of stock Giemsa to each cubic centimeter of water used. Usually 2 c.c. of fluid is sufficient to cover a slide completely. Ordinary tap water is satisfactory if slightly alkaline and low in mineral content. Allow blood smears to stain for at least thirty minutes, and tissue smears for as long as two hours. If dioxane is used it is not necessary to include the iodine step at all, since, as Guyer (p. 66) points out, dioxane dissolves bichloride of mercury. With dioxane the smear is passed through at least three changes of pure dioxane and then washed in water and stained as described. The mordant and stain may be conveniently applied by flooding the slides on a staining rack.

(2) *Tissues* dehydrated with *alcohols* were first washed for at least twelve hours in running water. They were then passed through the alcohols in the usual manner, including the iodine step, to absolute alcohol. Clearing and infiltration were done with toluene and absolute alcohol mixtures following the method of Galigher¹³ (1934, p. 122). Imbed and section as usual. Sections should be cut as thin as possible, 4 to 10 microns. The mounted paraffin ribbons were gently heated over an alcohol flame until just melted. The slide was immediately placed into two changes of pure xylene and then passed down through the alcohol series to water. Mordant and stain as for smears. All preparations should be allowed to stain for at least one hour. It is advisable to allow the mordant to remain for at least thirty minutes; longer periods will do no harm. When dioxane is used for dehydration, the tissues are immediately placed in dioxane without any preliminary washing. Change the dioxane at least once and imbed in paraffin, using the procedure of Guyer (p. 65). The mounted sections were heated over an alcohol flame as mentioned above, and then passed through two changes of pure xylene into dioxane. Wash in two changes of dioxane and transfer to water. Mordant and stain as previously described.

MOUNTING PROCEDURE

A. *The Alcohol-Acetone Method.* All Romanowsky-type stains are soluble in alcohol; it is this fact that makes the use of diaphane directly from 95 per cent alcohol impracticable, since a certain amount of the alcohol is carried over to the mounting medium, thus continuing the destaining process. This

eventually ruins the slide in as short a time as a week. In order to obviate this difficulty, the following technique was devised. It applies to both smears and tissues prepared by dehydration.

After staining, the preparations are dehydrated in an alcohol series. Destaining, if necessary, is carried out in 70 per cent alcohol. When a redder color is desired, the tissues may be placed for a few seconds in 95 per cent alcohol to which eosin has been added (1 drop of 0.5 Gm. eosin in 100 c.c. of 95 per cent alcohol to 10 c.c. of alcohol). Transfer from 95 per cent alcohol to solution A. Solution A is a mixture of 30 c.c. of 95 per cent alcohol, 30 c.c. of pure acetone, and 5 c.c. of diaphane. It is readily miscible. Destaining by the 95 per cent alcohol is stopped considerably in this mixture, but the slide should not be left in it longer than a minute. Transfer to solution B. This is a mixture of 55 c.c. of acetone, 10 c.c. of diaphane, and about 1 c.c. of 95 per cent alcohol. When the acetone and diaphane are mixed, a milky white solution results. But as the alcohol is added it clears up completely and remains so indefinitely. The alcohol is conveniently added drop by drop until the desired clear solution is obtained. This small amount of alcohol does not in any way cause destaining. Mount in diaphane.

Slides so prepared and mounted have remained without any evidence of fading for over a year. Preparations may be passed through solutions A and B directly from 70 per cent alcohol without any apparent damage to the tissues. The reason one cannot go straightway from 95 per cent alcohol to acetone to diaphane is that the solubility reaction between these substances is so drastic that the tissues usually crack and are ruined. The adding of diaphane to solutions A and B prevents the tissues from drying out quickly, since acetone is so volatile. It also prevents the acetone from going into solution too rapidly with the diaphane under the cover slip. The use of acetone with Romanowsky stains may be objectionable, but Kingsley¹⁴ (1937) showed that it was not detrimental to such stains.

Diaphane solvent, a preparation of secret formula marketed by some supply houses, may be used with equal success in solutions A and B in place of acetone. If desired, solutions of acetone and the solvent can be employed. Diaphane solvent was miscible in all proportions with 95 per cent alcohol, acetone, and dioxane. When preparations are placed in the undiluted solvent from 95 per cent alcohol, the mixing is rather slow and destaining is not stopped immediately. When the solvent is mixed in excess with 70 or 80 per cent alcohol, a milky white solution, similar to the one described for solution B, results. The addition of a small amount of 95 per cent alcohol clears this up. It is recommended, therefore, that if the diaphane solvent is to be used as the clearing agent, it should be prepared and employed as solutions A and B, substituting it for the acetone.

B. The Dioxane Method. Dioxane was found to be soluble in all proportions with diaphane. As would be expected, it does not destain preparations stained with any Romanowsky-type stains used. This makes it an ideal fluid in which to dehydrate such material for mounting in diaphane (or for that matter in balsam or cedar oil). The following procedure was utilized with much success:

Subsequent to staining, the slides are washed in water and destained, if necessary, in 70 per cent alcohol directly from the water wash. Fifty per cent alcohol works just as efficiently and has the advantage of being less drastic in action. Stop destaining by transferring quickly to pure dioxane. Pass through two changes of dioxane. Mount in diaphane. No clearing agent is necessary. However, if desired, graduated mixtures of diaphane and dioxane may be used to good advantage. Since the dioxane is not so volatile as acetone, it is best to remove some of the excess before mounting, thus allowing the slide to dry more quickly. Preparations mounted in this manner have the advantage over the alcohol-acetone method in that the destaining is stopped abruptly. This results in more uniformly stained tissues.

CONCLUSIONS

1. Diaphane is unconditionally recommended as a mounting medium for preparations stained with any Romanowsky-type stains in place of balsam or cedar oil.
2. An alcohol-acetone and a dioxane method suitable for dehydrating tissues stained with the Romanowsky-type stains are presented. Each method has been used successfully.
3. A simple method for staining tissues with Giemsa which has proved especially useful in bird malaria and fowl pox research is described.

REFERENCES

1. Lee, B.: *Microtometist's Vade Mecum*, Philadelphia, 1928, P. Blakiston's Son & Co.
2. Scott, S. G.: On Successful Double Staining for Histological Purposes, *J. Path.* 16: 390, 1911.
3. Guyer, M. F.: *Animal Micrology*, Chicago, 1936, Univ. Chicago Press.
4. Cannon, P. R., and Taliaferro, W. H.: Acquired Immunity in Avian Malaria. III. Cellular Reactions in Infection and Superinfection, *J. Prev. Med.* 5: 37, 1931.
5. Huff, C. G., and Bloom, W.: A Malarial Parasite Infecting All Blood and Blood-Forming Cells of Birds, *J. Infect. Dis.* 57: 315, 1935.
6. Kingsley, D. M.: A Technic for Megakaryocytes and Platelets in Sections, *Folia haemat.* 57: 87, 1937.
7. Wright, W. R.: A Note on Mounting Media for Parasitological Materials, *Ann. Trop. Med.* 21: 179, 1927.
8. Gilson, G.: Un nouveau médium solidifiable pour le montage des préparations microscopiques, *La cellule* 23: 427, 1906.
9. Shepherd, E. S.: A Substitute for Euparal, *Tr. Am. Mier. Soc.* 37: 131, 1918.
10. Groat, R. A.: Two New Mounting Media Superior to Canada Balsam and Gum Damar, *Anat. Rec.* 74: 1, 1939.
11. Wolbach, S. B.: Studies on Rocky Mountain Spotted Fever, *J. M. Research* 41: 1, 1919.
12. Hewitt, R.: A Staining Technic for Demonstrating Avian Malaria Parasites in Tissue Sections, *Am. J. Hyg. Sec. C* 29: 115, 1939.
13. Galigher, A. E.: *The Essentials of Practical Micro-technique in Animal Biology*, Berkeley, 1934, A. E. Galigher, Inc.
14. Kingsley, D. M.: Acetone Solvents for Romanowsky Stains, *J. LAB. & CLIN. MED.* 22: 524, 1937.

CHEMICAL

SIMPLIFIED PHOTOELECTRIC PROCEDURES FOR MICRO- DETERMINATION OF NONPROTEIN NITROGEN, UREA NITROGEN, CREATINE, AND CREATININE OF BLOOD*

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SIMPLE and accurate available microchemical methods for the measurement of the nonprotein nitrogen constituents of the blood of the albino rat require more blood than can be obtained from the living animal. The spectrophotometer methods of Borsook¹ could not be introduced, since our laboratory did not possess such an instrument. Furthermore, his procedures were considered too involved for routine use. The recently published electrotitration methods of Borsook and Dubnoff,² while yielding accurate results, were also considered too complex.

With the aid of the Evelyn Macro-Micro Photoelectric Colorimeter,³ a technique was developed for microdeterminations of nonprotein nitrogen, urea nitrogen, creatine, and creatinine. These procedures are so simple and accurate that they can be carried out by any intelligent hospital technician.

NONPROTEIN NITROGEN

Calibration curves were made using the 520 and 440 filters. The Koch and McMeekin⁴ procedure was used for the preparation of the Nessler-Folin reagent for microdeterminations. Instead of working on a 100 ml. basis, the technique adopted was developed on a tenth of a volume basis, i.e., a total dilution to 10 ml., which was carried out in 15 ml. centrifuge tubes graduated in tenths of a milliliter. For the digestion an electrically heated microapparatus, purchased from Eimer and Amend, was used. The Kjeldahl flasks had a total capacity of 10 ml. Tungstic acid blood filtrates were prepared according to the method of Folin,⁵ taking the blood with 7 volumes of ammonia-free water, and precipitating the proteins with 1 part of 10 per cent sodium tungstate made up fresh daily, and 1 part of $\frac{2}{3}$ normal sulfuric acid.

Procedure.—Measure 0.5 ml. of blood filtrate with a 0.5 ml. delivery pipette into a 10 ml. Kjeldahl flask and add 1 ml. of 1:20 sulfuric acid. Digest until fumes appear and add 1 drop of superoxol. Insert a small funnel against the

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fume receiver. Heat again until fumes appear, then heat for one minute, timing with a stop watch. Cool and dilute with about 5 ml. of ammonia-free distilled water. Wash out flask with additional distilled water into a 15 ml. graduated centrifuge tube up to 8.5 ml. mark. Then add 1.5 ml. of the Nessler-Folin reagent.⁵ Shake quickly by pouring back and forth from centrifuge tube to absorption tube three times. By such procedure clouding is avoided. Set the blank with reagents at 100 and then read in Evelyn Photoelectric Macro Colorimeter, using the 520 filter, and obtain values from the calibration curve (Fig. 1). The determinations are made in duplicate or triplicate.

The accuracy of the method was tested on two different samples of urine, one on rat's urine and the other on man's urine, using the standard macro-Kjeldahl method for comparison. The results are given in Table I. It is evident from these data that our microprocedure checks to the extent of 1 to 2 per cent with the macro-Kjeldahl method.

TABLE I

URINE	VOLUME (ML.)	NUMBER OF DETERMINATIONS	METHOD	NITROGEN MG. PER ML.	PER CENT ERROR
Rat	0.001	0	Sure and Wilder	58.0	
	1.0	3	Macro-Kjeldahl	58.5	+0.80
	6.0	3	Macro-Kjeldahl	59.0	+1.72
Man	0.02	8	Sure and Wilder	11.52	
	1.0	3	Macro-Kjeldahl	11.76	+2.09

TABLE II

UREA NITROGEN USED FOR ANALYSIS (μ G)	UREA NITROGEN FOUND (μ G)	PER CENT RECOVERY
20.0	20.00	100.00
20.0	20.05	100.25
30.0	31.00	103.30
30.0	32.00	106.60
40.0	41.00	102.50
40.0	30.00	97.50
40.0	39.00	97.50
50.0	52.00	104.00

UREA NITROGEN

To 0.5 ml. of blood filtrate add 2 drops of a phosphate buffer solution⁷ and two drops of a 1 per cent filtered urease solution freshly prepared from Squibb's urease powder. Let stand for one hour at room temperature. Dilute to 7.5 ml. in a 15 ml. centrifuge tube and add 1 ml. of 1:20 sulfuric acid. Transfer from centrifuge tube to absorption tube back and forth twice and shake. This is done to avoid subsequent clouding following addition of the Nessler-Folin reagent. Now add 1.5 ml. of the Nessler-Folin solution. Transfer back and forth to absorption tube three times and shake. Set blank with reagents at 100 and read in photoelectric colorimeter, using the 440 filter, and obtain values from calibration curve (Fig. 1). Recoveries of urea nitrogen by the above micromethod are given in Table II. The nesslerization of the various duplicate or triplicate samples should be done one at a time to prevent clouding.

It is apparent from the results in Table II that our micro urease method yields satisfactory recoveries from known urea solutions. The average error seems to be not greater than ± 3.0 per cent.

CURVES FOR NON-PROTEIN NITROGEN
OF BLOOD
EVELYN MACRO PHOTOELECTRIC COLORIMETER

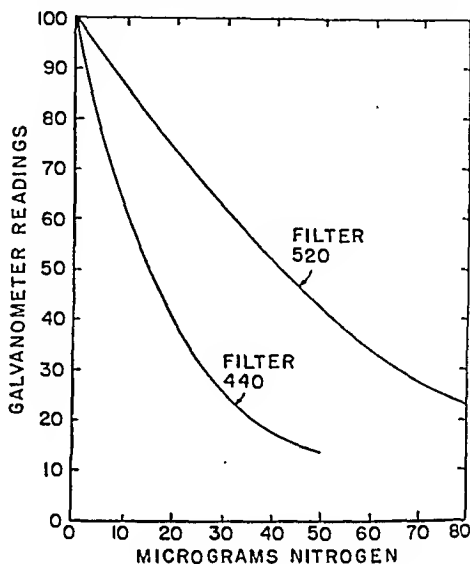


Fig. 1.

CURVES FOR PREFORMED AND TOTAL CREATININE
OF BLOOD
EVELYN MICRO PHOTOELECTRIC COLORIMETER

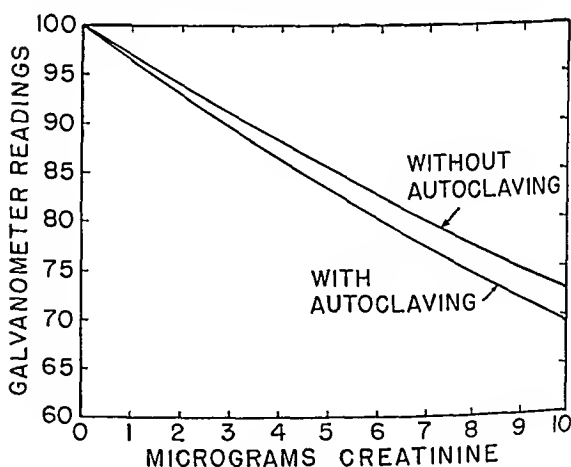


Fig. 2.

PREFORMED CREATININE

The Evelyn Micro Photoelectric Colorimeter was used. The ordinary cadmium-coated plungers corroded with saturated picric acid; therefore, gold-plated plungers made by the Rubicon Company of Philadelphia, manufacturers of the Evelyn Colorimeter, were introduced. The 520-M filter was used.

Procedure.—To 1 ml. of blood filtrate add 0.5 ml. of saturated picric acid solution and let stand for ten minutes. Then add 0.1 ml. of 10 per cent carbonate-free sodium hydroxide. After setting the blank with reagents at 100, read the microcolorimeter and obtain values from the calibration curve (Fig. 2).

All attempts to convert creatine to creatinine with either sulfuric or hydrochloric acid, using the micro cells of the Evelyn Photoelectric Micro Colorimeter, yielded irregular results, and such procedure, therefore, had to be abandoned. The following technique was found very satisfactory: To 1 ml. of blood filtrate add 0.5 ml. of saturated picric acid in a 15 ml. centrifuge tube. Cover with lead foil. Autoclave for forty minutes at 20 pounds pressure. Cool. Add 0.1 ml. of 10 per cent carbonate-free sodium hydroxide and allow to stand for ten minutes. Blank with reagents is also autoclaved for the same time at the same pressure. After setting blank at 100, read in the microcolorimeter and obtain values from a separately calibrated curve (Fig. 2). The plungers should be allowed to be in contact with the picric acid not more than a minute or two and then quickly removed from the cell, rinsed with distilled water, and dried. This is done because of the possibility of corrosion by being allowed too much contact with the saturated picric acid solution, particularly since the gold plating is very thin. The difference between the values for the total creatinine and the pre-formed creatinine represents the creatine expressed as creatinine.

Recovery of creatinine from creatine hydrate (Eastman Kodak Co.) is given in Table III.

TABLE III

CREATINE HYDRATE USED (μ g)	NUMBER OF DETERMINATIONS	THEORETICAL AMOUNT OF CREATININE TO BE RECOVERED (μ g)	ACTUAL CREATININE RECOVERED (μ g)	PER CENT ERROR
10.0	6	7.51	7.45	-0.79
5.0	7	3.76	3.85	+2.66

It is evident from the above results that the recovery of creatinine from pure creatine by the simplified technique described is almost complete, the experimental error ranging between -0.79 and +2.66.

Total blood creatinine was determined in 16 rats of different ages from our stock colony, the results ranging from 6.7 to 8.65 mg. per 100 ml. of blood. The average was 7.55 mg. per cent.

SUMMARY

Simplified technique is described for the microdeterminations of nonprotein nitrogen, urea nitrogen, creatine, and creatinine in the blood with the aid of the Evelyn Macro-Micro Photoelectric Colorimeter. This technique can be applied to capillary blood of small laboratory animals as well as human beings.

REFERENCES

1. Borsook, H.: Micromethods for Determination of Ammonia, Urea, Total Nitrogen, Uric Acid, Creatinine (and Creatine), and Allantoin, *J. Biol. Chem.* 110: 421, 1935.
2. Borsook, H., and Dubnoff, J. W.: Methods for the Determination of Submicro Quantities of Total Nitrogen, Ammonia, Amino Nitrogen, Amides, Peptides, Adenylic Acid, and Nitrates, *J. Biol. Chem.* 131: 163, 1939.

3. Evelyn, K. A.: Stabilized Photoelectric Colorimeter With Light Filters, *J. Biol. Chem.* 115: 63, 1936.
4. Evelyn, K. A.: Photoelectric Microcolorimeter, *Ibid.* 117: 365, 1937.
5. Koeh, F. C., and McMeekin, T. L.: A New Direct Nesslerization Micro-Kjeldahl Method and a Modification of the Nessler-Polin Reagent for Ammonia, *J. Am. Chem. Soc.* 46: 2066, 1924.
6. Folin, O.: *Laboratory Manual of Biological Chemistry*, New York, 1926, D. Appleton-Century Company, p. 227.
7. Peters, J. P., and Van Slyke, D. D.: *Quantitative Clinical Chemistry*, Baltimore, 1932, Williams & Wilkins Co., Vol. II, Methods, p. 547.

CLINICAL APPLICATION OF THE MICRODIFFUSION METHOD FOR THE ESTIMATION OF ACETONE*

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IN A RECENT publication¹ regarding the microdiffusion method for the estimation of acetone, the theory and work on which the method is based was discussed. Reference was also made to the clinical application of the method. The purpose of this paper is to offer a rapid procedure which may be used in a clinical laboratory. A comparison is also made between results obtained by this method and by the colorimeter method of Behre and Benedict.²

APPARATUS AND TECHNIQUE

The microdiffusion apparatus is the Conway unit. This unit consists of a small dish of fairly thick glass, resembling a Petri dish (Fig. 1).³ It is divided into two chambers by a circular wall of glass arising from the floor of the dish to about half the height of the outer wall. When in use it is covered with a transparent glass plate and sealed with a fixative.

The Deniges reaction⁴ of acetone precipitation as a basic mercuric compound is utilized. The factors concerned are the time necessary to cause precipitation and the temperature at which the reaction takes place. Two cubic centimeters of Nessler's solution, three cubic centimeters of unknown, and one cubic centimeter of 1 per cent hydrochloric acid are required. Since acetone is volatile, the unknown (urine or oxalated whole blood) should be stoppered as soon as collected and used fresh. The acid is used to fix any ammonia which may be present in the unknown.

Carefully cleaned units are obligatory. This may be accomplished by dipping into cleaning solution and then rinsing consecutively with water, alcohol, and ether.

When ready for use, the units with their covers are placed on a black background, and the edges of the outer wall are smeared with vaseline. Next, the reagents are introduced. First, the central chamber receives the Nessler's solution, and then the acid is run in, distributing it evenly around the outer chamber. The transparent glass plate is next placed to cover the entire unit, except for a small slit, permitting the introduction of the end of the pipette used to carry the unknown—urine or oxalated whole blood. A stop watch held in one hand is

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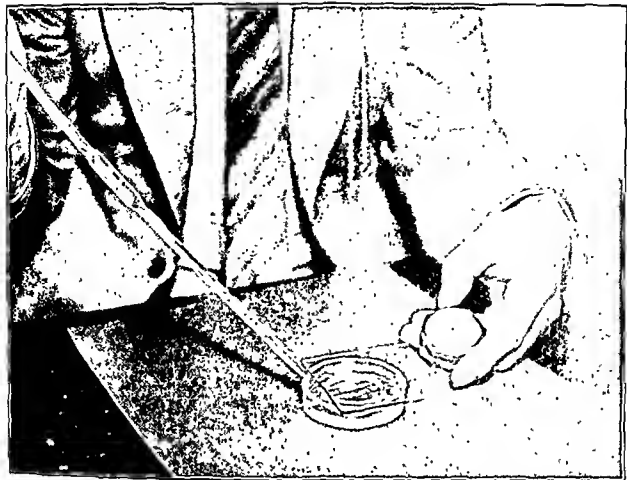
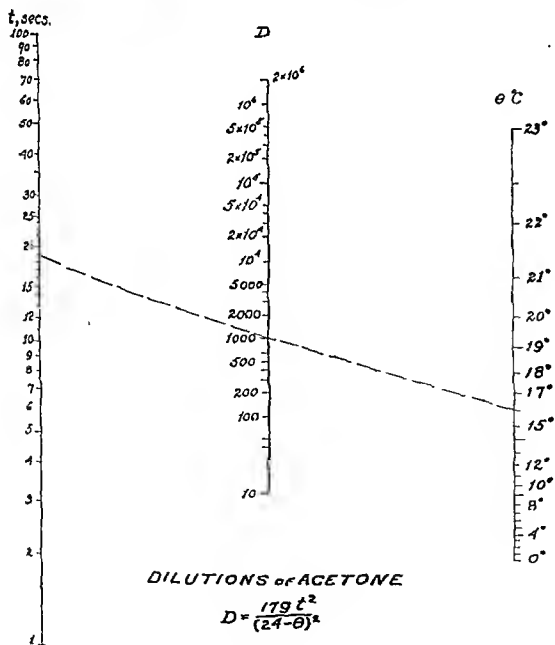


Fig. 1.

NOMOGRAPHIC CHART



started as soon as the pipette containing the unknown, held by the other hand, stops flowing freely. The hand holding the watch next slides the glass plate so that it completely covers the unit, as an immediate gesture after starting the watch. When the endpoint is reached the watch is stopped. Rapid introduction of the unknown is best done with a pipette possessing a large opening. The endpoint is the appearance of precipitation of the Nessler's solution at the periphery of the inner chamber of the unit in the form of threads, like spokes of a wheel, or as a cloud. It is that second when the spokes or cloud extend inward 1 mm.

The temperature factor may be controlled without great loss of accuracy by placing the reagents and utensils in a cool (20° C.) corner of the laboratory for an hour. The reading on a thermometer hung close by may be used. Time readings varying no more than ± 2 seconds from three tests may be averaged and considered sufficient. The temperature and time factors thus obtained are then located on the nomogram (Fig. 2) with a straight edge, and the concentration of acetone is read off in the center.

COMPARISON WITH BEHRE-BENEDICT METHOD

It was considered well to compare this method with another of proved value.

The following dilutions of specified acetone in human urine and oxalated ox blood were made from a 1 per cent solution and estimated by the two methods: 1:500, 1:1,000, 1:3,000, 1:7,000, and 1:10,000. Incubation of the apparatus and reagents was again resorted to in our method in order to control better the temperature factor. Table I shows the values obtained.

TABLE I
ACETONE IN MILLIGRAMS PER 100 C.C. OF URINE OR BLOOD

DILUTIONS	ACETONE IN HUMAN URINE		ACETONE IN OXALATED WHOLE OX BLOOD	
	MICRODIFFUSION METHOD	BEHRE-BENEDICT METHOD	MICRODIFFUSION METHOD	BEHRE-BENEDICT METHOD
1:100	95.00	98.00	90.00	95.00
1:500	19.50	19.50	19.00	19.50
1:1,000	10.00	9.50	11.00	9.50
1:3,000	3.50	3.00	3.00	3.50
1:7,000	1.50	2.00	1.50	2.50
1:10,000	1.20	1.50	1.30	1.80

SUMMARY

The microdiffusion method for the estimation of acetone has been modified to fit the needs of the clinical laboratory. With a little practice a urine or blood sample may be determined in five to ten minutes.

This work was begun at Trinity College, Dublin, under the direction of Professor W. R. Fearon, of the Department of Biochemistry. I wish to thank him for his interest and kind assistance. I also wish to thank Mr. Einhart Kameran, who took part in the development of the technique.

REFERENCES

1. Werch, S. C.: A Microdiffusion Method for the Estimation of Acetone, *J. LAB. & CLIN. MED.* 25: 414, 1940.
2. Behre, J. A., and Benedict, S. R.: Colorimetric Method for the Estimation of Acetone, *J. Biol. Chem.* 70: 487, 1926.
3. Gallenkamp, A., and Co., Ltd., 17-29 Sun Street, Finsbury Square, London, E. C. 2.
4. Deniges, F.: *Précis de Chimie Analytique*, ed. 6, Lyon, 1930, A. Stork & Cie, p. 213.

A CLINICAL METHOD FOR EXTRACTION OF URINARY ANDROGENS PRELIMINARY TO COLORIMETRIC QUANTITATION BY OESTING'S TECHNIQUE*

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ALL those engaged in the quantitation of urinary androgens have felt the need of a method less expensive and requiring a shorter period of time for completion than those of bio-assays which employ capons or baby chicks. It was with enthusiasm, therefore, that clinicians and research workers turned to the reports of Zimmermann for a chemical method for the determination of urinary androgens.

Zimmermann demonstrated^{1, 2} that the steroid sex hormones could be determined quantitatively by the use of meta-dinitrobenzene by means of a characteristic color reaction which occurred in the presence of an alkali with compounds containing an active methylene group. Since his work was done chiefly with crystalline sex steroids, an application of the "Zimmermann reaction" had to be devised for urinary extracts.

Several such methods had been described as suitable for the determination of urinary androgens when the procedure described herein was initiated.³⁻⁵ Careful consideration was given to these methods, with particular regard to the expense of new equipment necessary, the relative simplicity of the technique, the time elements involved, and the practicality of doing consecutive daily assays on a number of patients. Oesting's method⁴ seemed to satisfy best our requirements.

The method for extraction used by Oesting is essentially that of Gallagher and Koch.⁶ In their procedure benzol was the solvent employed and the extraction was carried out in a continuous extractor. We have modified the method of extraction so as to eliminate the use of benzol and the continuous extractor. Although the latter is very efficient, it is of such dimensions as to be impracticable in the smaller clinical laboratories. Because of its high toxicity and inflammability, benzol is a dangerous solvent to use in the average laboratory space.

The extracting medium employed in the procedure described below is dibutyl ether.⁷ Besides being a thoroughly satisfactory solvent⁸ for steroids, dibutyl ether has a number of advantages over other solvents. It is practically nontoxic and is nonirritating to the mucosa of the air passages. It has a low kindling

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¹Secured from the Union Carbide and Carbon Corporation, Long Island City, New York.

point, and, a fact which is very important, urinary pigments are only slightly soluble in it. The extractions can be made in a simple apparatus. The recovery after distillation is approximately 90 per cent, and the solvent can be used a second time before redistillation is necessary.

METHOD OF EXTRACTION

An aliquot fraction of a twenty-four-hour specimen of urine (not less than 200 c.c., if possible) is acidified, using 5 c.c. of concentrated sulfuric acid (reagent) per 100 c.c. of urine. Hydrolysis is accomplished by autoclaving for fifteen minutes at 15 pounds pressure. When cool the urine is extracted three times with dibutyl ether. Fresh solvent is used for each extraction. For specimens which are more than 500 c.c. in volume, 20 c.c. of solvent per 100 c.c. are used; for specimens of less than 500 c.c. in volume, only two extractions are made, using 150 c.c. of solvent for each extraction. The extraction is carried out in thin-walled, globe-shaped 2L separatory funnels by violent shaking by hand at the rate of 180 shakes per minute for three minutes for each extraction.

The combined dibutyl ether extracts are washed with (1) 100 c.c. of 10 per cent sodium hydroxide, (2) 100 c.c. of sodium bicarbonate solution saturated at room temperature, and (3) 100 c.c. of tap water. These amounts are used when the amount of dibutyl ether extract is 300 c.c. or less. Proportionately more of the solutions must be used if the volume of dibutyl ether extract is greater than 300 c.c. (By washing the dibutyl ether extract with the two alkalis, the estrogens are partially removed. These are completely removed in a subsequent step. The strong alkali breaks down the emulsion which sometimes had formed and removes most of the pigment which had been extracted by the solvent.)

The washed dibutyl ether extract is then transferred to a 2L distillation flask, and the solvent is removed by steam distillation under reduced pressure. When dry the flask is washed out three times with ethyl ether, using a total of 100 c.c. (Anesthetic ether is used in order to employ a product as nearly peroxide and aldehyde free as possible.)

The ethyl ether extract of the dry residue is transferred to a 125 c.c. separatory funnel where it is washed with 20 c.c. portions of 10 per cent sodium hydroxide until the washings are clear. This usually requires three to five washings, occasionally more. The ethyl ether extract is then washed three times with 20 c.c. of distilled water.

The ethyl ether extract is transferred to a 250 c.c. beaker containing approximately 0.5 Gm. of decolorizing charcoal.* The solution is thoroughly stirred, and the charcoal is allowed to settle out before filtering. The charcoal is well washed with additional ether. To complete the final step in the extraction of the urinary androgens, the ether is allowed to evaporate or it is evaporated to dryness over a steam bath.

Occasionally faulty filter paper will allow the passage of some charcoal. This cannot be observed until the ether is all evaporated. In this event the extract should be taken up in 100 c.c. of ether and refiltered. Although discoloration due to charcoal is obvious, there occasionally appears a brownish residue in the beaker after evaporation of the ether, for

*"Norit-A," purchased from the Arthur H. Thomas Co., Philadelphia, Pa.

which no explanation is offered. This brown residue is difficult to remove by further treatment with charcoal. Moreover, additional treatment with charcoal and subsequent filtering so greatly reduces the steroid content of the ether extract that subsequent colorimetric determinations are valueless.

New lots of charcoal should be checked against the charcoal being used with regard to steroid adsorption and general quality of the powder. One brand of charcoal allowed no measurable amount of steroids to pass through the filter. Other brands have given trouble with regard to colored or muddy-appearing residues after evaporation of the ether extract.

COLORIMETRIC DETERMINATION

No changes have been made in the colorimetric determination part of Oesting's procedure. The colorimeter used is one designed by Oesting with the assistance of the Hellige Manufacturing Co.

It has been our constant practice that one of us (W.K.C.) attend to the extraction of specimens and the other (M.B.) carry out the colorimetric procedure. We believe that in this manner personal error has been minimized.

The following data, representing 220 colorimeter readings, illustrate the accuracy which is obtained by careful technique:

110 determinations made in duplicate
68 of these, or 62 per cent, matched
42 of these, or 38 per cent, did not match

Of the 42 which did not match
15 differed by only 0.1 color unit
9 differed by only 0.2 color unit
7 differed by only 0.3 color unit
8 differed by only 0.4 color unit
2 differed by only 0.5 color unit
1 differed by only 0.7 color unit

Although this method does not permit qualitative identification of any member of the androgen group, it is felt that these data on total "androgens" as a group (17-ketosteroids) are of very definite value. This value is enhanced by consecutive daily determinations. It has been shown by our group that there is a marked variation in the daily excretion of these compounds by women.⁹ These variations take on some significance in view of the apparent accuracy of the method.

SUMMARY

A clinical method for extraction of urinary androgens, preliminary to colorimetric quantitation, has been described. Dibutyl ether is substituted for benzol as the solvent. The advantages of dibutyl ether are enumerated.

The method of extraction is adaptable to the average laboratory and is practicable for assaying consecutive daily specimens on a number of patients concurrently.

We wish to express our indebtedness and thanks to Dr. E. C. Hamblen, Chief of Endocrine Division, for his constant support and helpful criticism.

REFERENCES

1. Zimmermann, W.: Eine Farbreaktion der Sexualhormone und ihre Anwendung zur quantitativen colorimetrischen Bestimmung, *Ztschr. f. physiol. Chem.* 233: 257, 1935.
2. Zimmermann, W.: Colorimetrische Bestimmung der Keimdrüsenhormone, *Ztschr. f. physiol. Chem.* 245: 47, 1936.

3. Wu, H., and Chou, C.-Y.: Colorimetric Methods for the Determination of Sex Hormones in Human Urine, *Chinese J. Physiol.* 11: 413, 1937.
4. Oesting, R. B.: Colorimetric Assay for Male Sex Hormones in Urine, *Proc. Soc. Exper. Biol. & Med.* 36: 524, 1937.
Oesting, R. B.: Colorimetric Assay of Urinary Androgens, *Proc. Soc. Exper. Biol. & Med.* 39: 76, 1938.
5. Callow, R. K., and Emmens, C. W.: Colorimetric Determination of Substances Containing Grouping— CH_3CO —in Urine Extracts as Indication of Androgen Content, *Biochem. J.* 32: 1312, 1938.
6. Gallagher, T. F., and Koch, F. C.: Quantitative Assay for Testicular Hormone by Comb-Growth Reaction, *J. Pharmacol. & Exper. Therap.* 55: 97, 1935.
7. McCullagh, D. R., and McLin, T. R.: Extraction of Androgens From Urine, *Endocrinology* 22: 120, 1938.
8. McCullagh, D. R., and Guillet, R.: Summary of Some Laboratory Methods in Endocrinology, *Cleveland Clin. Quart.* 6: 95, 1939.
9. Hamblen, E. C., Ross, R. A., Cuyler, W. K., Baptist, M., and Ashley, C.: Studies on the Metabolism of Androgens in Women, *Endocrinology* 25: 491, 1939.

SYRINGE TYPE GLASS ELECTRODE FOR THE MEASUREMENT OF BLOOD PH*

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MANY glass electrodes have been designed for measuring the pH of blood as it exists in the body, but there are relatively few that are free from objections. To be satisfactory for blood analysis, the electrode not only must conform to the usual standards for glass electrodes but also must meet the following additional requirements:

- (1) Analysis of small samples of blood should be possible.
- (2) The blood must not be altered by coming into contact with air or by getting mixed with another solution.
- (3) The blood should not undergo marked changes in temperature.
- (4) The pH of the blood must be measured quickly, before glycolysis sets in.
- (5) The blood should be obtained without stasis and without the use of suction by means of a syringe.

In our opinion, the electrode described here† meets all these requirements and has additional features which make it suitable for other studies. We have used it for over a year with very satisfactory results.

The drawing in Fig. 1 shows the construction of the electrode. It is a modification of the type developed by MacInnes and Belcher¹ in which the glass membrane is well protected because it forms the inner tube of the electrode. The tip at A is ground to fit a standard Luer hypodermic needle. The ground surface at B makes it possible to seal the electrode with a glass stopper, or to

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†Obtainable from Mr. Otto Hopf, Flushing, N. Y.

introduce the tip of a standard syringe so that the electrode forms an extension of the barrel of the syringe. The over-all length of the electrode is 9 cm., and only 0.5 c.c. of blood is necessary to fill it completely. At *C* a reference solution and electrode are introduced; we have used 0.1 N hydrochloric acid and a silver-silver chloride electrode,² sealed into place with De Khotinsky cement. The electrode is rugged and convenient to handle; furthermore, the straight glass membrane can be easily cleaned by means of a soft feather and water.

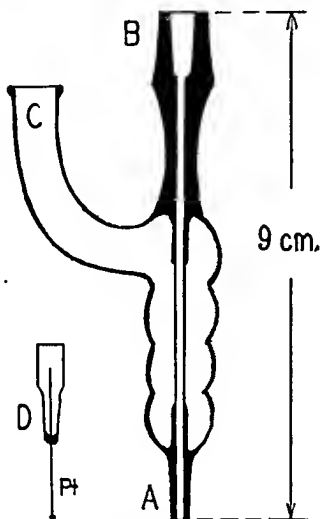


Fig. 1.—Syringe type glass electrode and special plug for measurement of oxidation-reduction potentials.

The measurements were made with a Beckman pH meter which permitted readings accurate to 0.03 pH units. To insure constant temperature the glass electrode and calomel half cell are hung in a saturated solution of potassium chloride which is kept at the desired temperature by circulating water. A sketch of the small water bath which was constructed to fit the Beckman pH meter is shown in Fig. 2. A simple brass clip attached to a bakelite block (Fig. 2) insulates the glass electrode from the pH meter and holds it in place. The potassium chloride solution serves further as a liquid junction between the glass electrode and the calomel half cell.

Often it is desirable to measure oxidation-reduction potentials as well as the pH of the same solution. If instead of the glass stopper, a special plug (Fig. 1, *D*) is used into which a platinum wire has been sealed, both measurements can be carried out on the same sample and with the same meter.

Operation of the Electrode.—The electrode is warmed to the desired temperature and calibrated with several buffers of known pH. It is advisable to use buffers with pH values near that of the unknown. When repeated meas-

urements prove the satisfactory performance of the electrode, it is washed in the warm circulating water and rinsed with a warm solution of heparin in physiologic saline solution. A sterile hypodermic needle is then attached to tip A and the blood vessel is punctured. During the sampling the electrode temperature is kept near body temperature by holding it covered with the hand. If arterial blood is taken, the electrode will fill very quickly because little resistance is offered by the straight barrel of the electrode to the flow of blood.

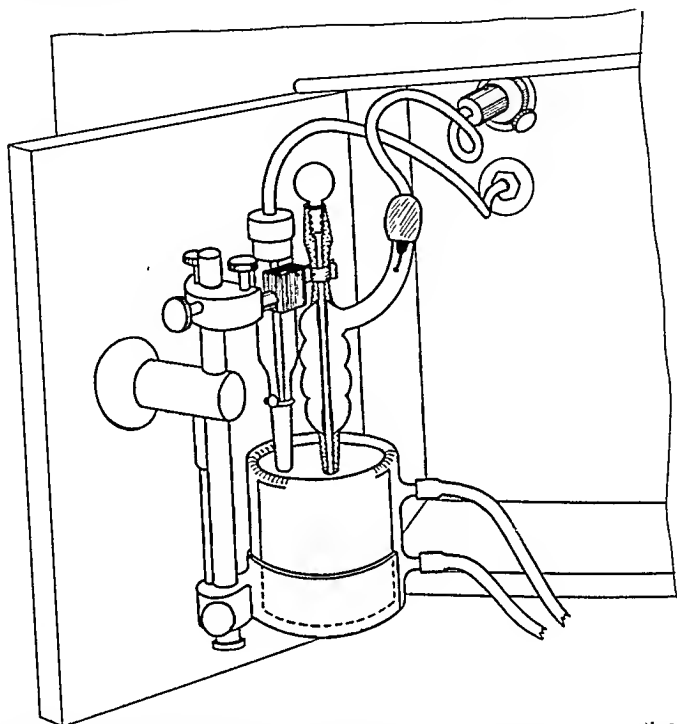


Fig. 2.—Sketch showing glass electrode, calomel half cell, and water bath attached to door of pH meter. Raising the water bath will immerse the electrodes in a saturated solution of potassium chloride which serves as liquid junction.

If venous blood is taken, the electrode has to be held in a horizontal position, or slight stasis of the vein becomes necessary to force the blood through the electrode. In addition to the 0.5 c.c. which is necessary to fill the electrode, another 0.5 c.c. of blood is passed through the electrode. This washes out any excess heparin so that the composition of the blood should be the same as that in the blood vessel. While the blood is still flowing, the electrode is stoppered. It is then withdrawn and hung into the potassium chloride solution after the needle has been removed. Stable potentials are obtained immediately. The whole procedure requires less than one minute.

The pH values obtained are reproducible if the blood samples are taken from the same person within an hour. In agreement with the observations of others,^{3, 4} we found that the presence of heparin does not have a measurable effect on the pH; neither does clotting alter the observed pH⁵. However, if the blood is left in the electrode more than five minutes, a drift to a lower pH, due to glycolysis, is noticeable.^{4, 5} Some studies made with this electrode on the blood pH of normal and hypertensive dogs are published elsewhere.⁶

REFERENCES

1. MacInnes, D. A., and Belcher, D.: A Durable Glass Electrode, *Indust. & Engin. Chem. Anal. Ed.* 5: 199, 1933.
2. Brown, A. S.: A Type of Silver Chloride Electrode Suitable for Use in Dilute Solutions, *J. Am. Chem. Soc.* 56: 646, 1934.
3. Yoshimura, H.: A New Micro-Glass Electrode and the pH of Arterial, Venous and Capillary Blood, *J. Biochem.* 23: 335, 1936.
4. Haugaard, G., and Lundsteen, E.: Über die Messung des pH im Blute mit Hilfe der Glaselektrode, *Biochem. Ztschr.* 285: 270, 1936.
5. Ferguson, J. H., and DuBois, D.: Observations on the pH of Clotting and Citrated Blood, *J. LAB. & CLIN. MED.* 21: 663, 1936.
6. Muller, O. H., and Nickel, W. F.: The pH of Systemic Blood in Normal and Hypertensive Dogs, Determined by Means of a Syringe Type Glass Electrode, *Proc. Soc. Exper. Biol. & Med.* 43: 89, 1940.

COMPARATIVE STUDY ON A NEW SIMPLE METHOD OF SULFANILAMIDE DETERMINATION IN BLOOD*

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THE extensive use of sulfanilamide treatment and the recognized importance of attaining and maintaining an optimal blood concentration make it very desirable to have a method which combines simplicity and accuracy in estimating this blood concentration.

In this country the method developed by Marshall (1937) is accepted as satisfactory. Recently, Werner (1939) described in *Lancet* a novel method for which he claims advantages over Marshall's diazotization reaction; namely, simplicity of technique, which results in a considerable saving of time, and the use of only reliable reagents, since the new method eliminates the use of the unstable coupling agent, dimethylnaphthylamine. This method is based on the direct formation of a yellow color when Ehrlich's reagent, para-dimethylamino-benzaldehyde, is added to the filtrate from blood containing sulfanilamide.

There has been no report of investigation of this method in American literature. The purpose of this publication is to compare Werner's method with that of Marshall.

About fifty determinations of free sulfanilamide in blood in varying cases have been made by this method. The following procedure incorporating some modifications was found satisfactory: 2 c.c. of oxalated blood are added drop by drop from a pipette to 8 c.c. of a 5 per cent solution of trichloroacetic acid. The blood protein is thereby immediately precipitated and is easily filtered off, yielding a clear filtrate. Next 3 c.c. of fourth-normal sodium hydroxide is added to 4 c.c. of filtrate, followed by 1 c.c. of Ehrlich's reagent. The yellow color develops immediately to its maximal intensity. It is compared in the colorimeter to the color developed by adding 1 c.c. of the reagent to 7 c.c. of a standard solution of sulfanilamide in distilled water, or by diluting 1.25 c.c. of the reagent to 10 c.c. with the standard. The standard, containing 0.08 mg. of sulfanilamide

*From the Pathological Laboratory of St. Vincent's Hospital, Norfolk.
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per 7 c.c. of water, is conveniently prepared by dissolving 0.8 Gm. of sulfanilamide in 700 c.c. of water, or 1.143 Gm. of sulfanilamide in 1,000 c.c. of water and diluting 1:100.

If the unknown is set at 10 in the colorimeter, the readings of the standard will be milligrams per hundred cubic centimeter of blood. When the concentration of the unknown exceeded 10 mg., exact results were not obtained. However, if the unknown is set at 5 and the readings of the standard are multiplied by 2, the results are exactly accurate.

The reagents used include: 5 per cent trichloroacetic acid, Ehrlich's reagent (3 Gm. of para-dimethylaminobenzaldehyde added to 100 c.c. water containing 3 c.c. of concentrated sulfuric acid), and fourth-normal sodium hydroxide.

TABLE I

MG. SULFANILAMIDE IN 100 C.C. BLOOD	AVERAGE RESULTS BY METHOD OF:	
	WERNER	MARSHALL
2.5	3.1	2.5
5.0	5.1	5.0
7.2	7.0	6.8
9.0	8.9	8.9
10.3	10.6	10.1
13.0	13.0	12.9
15.0	14.9	14.8

Table I shows the results obtained by Marshall's method compared with those obtained by Werner's method. Examination of blood to which sulfanilamide has been added to produce known concentrations showed essentially identical results.

Examination of the blood of sulfanilamide-treated patients likewise yielded the same results by both methods. There has not been any interference by other medications given to the patient.

Werner's method is accomplished by an extremely simple procedure which seems to make it superior for large scale use and saves considerable time for a single determination. The time required for one test by Marshall's method was about fifty minutes, although only fifteen minutes of that time was spent in actual manipulation. A determination by Werner's method can be completed in twelve minutes. The stability of the color developed by this procedure makes possible the use of a single standard solution for a large number of determinations. It fails to show appreciable change after two weeks' time. Ample time is available for reading the tests, and a number may be done simultaneously and read when convenient. The color formed by Marshall's procedure deteriorates rapidly, and each test must be read at a definite time, eliminating the possibility of carrying out many tests entirely simultaneously. A new standard must be made at least for each series of determinations.

Although the dimethylaminobenzaldehyde solution has a slight yellowish color, the distortion caused by it is not significant, except at very low concentrations (Table I). If the dimethylaminobenzaldehyde crystals are very old and show a greenish-brown color, the resulting solution is too yellow for use.

Werner's method is equally satisfactory for sulfapyridine determination. A sulfapyridine standard may be made or the sulfanilamide standard used and the result multiplied by 1.5 to correct for differences in molecular weight.

Werner also describes a very simple procedure for the determination of sulfanilamide in urine. He further gives directions for making a permanent set of standards from potassium chromate for use in a color comparator. Their use did not prove to be very satisfactory.

A disadvantage of Werner's method is that the yellow color developed is more difficult to compare in the colorimeter than the reddish one obtained by Marshall's procedure.

SUMMARY

A new method for determination of sulfanilamide in blood developed by Werner has been compared with that of Marshall. This method gives accurate results while allowing a considerable saving of time. The reagents used, as well as the standard solution, are stable.

The disadvantage of this method is its use of yellow for colorimetry.

REFERENCES

- Marshall, E. K., Jr.: Determination of Sulfanilamide in Blood and Urine, *J. Biol. Chem.* 122: 263, 1937.
 Marshall, E. K., Jr., Cutting, W. C., and Emerson, K., Jr.: Acetylation of Para-Aminobenzenesulfonamide in Animal Organism, *Science* 85: 202, 1937.
 Marshall, E. K., Jr., Emerson, K., Jr., and Cutting, W. C.: Para-Aminobenzenesulfonamide; Absorption and Excretion; Methods of Determination in Urine and Blood, *J. A. M. A.* 108: 953, 1937.
 Werner, A. E. A.: Estimation of Sulfanilamide in Biological Fluids, *Lancet* 1: 18, 1939.

RAPID VOLUMETRIC DETERMINATION OF URINARY SODIUM*

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RECENTLY Strauss¹ demonstrated that urine, to which has been added thorium nitrate and sulfuric acid, can be ashed very rapidly in silica beakers in an electric muffle furnace. The method was developed primarily for the determination of potassium and, although the author suggested that it might be adapted to the determination of sodium, she did not establish the fact that this could be done. Her method is much more convenient than the older one of Stolte² as applied by Tisdall and Kramer³ which is both tedious and time-consuming.

We wished, in this laboratory, to make a large number of sodium determinations and none of the methods now available seemed suited to our needs because of the time they required. We, therefore, decided to try to combine the rapid ashing technique of Strauss with the determination of sodium as the pyroantimonate by the method of Kramer and Gittleman,⁴ and we found that such a combination was very satisfactory. With these combined techniques one may determine sodium in duplicate on as many as eight urine samples in about five hours of elapsed time, or about three hours of actual work.

*From the Research Laboratories, Winthrop Chemical Co.
 Received for publication, April 15, 1940.

Reagents.—All solutions are prepared as described in the original articles with one exception: the solution used to extract the ash must be 0.4 per cent phosphoric acid instead of the 0.1 per cent solution recommended by Strauss. The reasons for this will be set forth below. This reagent, 10 per cent thorium nitrate, 10 per cent potassium hydroxide, and potassium pyroantimonate reagent are kept in paraffin-lined bottles.

Procedure.—An amount of urine containing 15 to 25 mg. of sodium (ordinarily this would be 5 to 7 c.c.) is measured into a silica beaker of 50 or 100 c.c. capacity, together with 1 c.c. of 10 per cent thorium nitrate and 1 c.c. of 4 N sulfuric acid. The material is evaporated on the water bath, ashed and extracted (with 0.4 per cent phosphoric acid), as described by Strauss. Samples of 1 c.c. of the extract are taken for analysis. To these samples are added 4 drops of 10 per cent potassium hydroxide to neutralize the phosphoric acid, after which the procedure followed is that of Kramer and Gittleman as modified by Eisenman.⁵

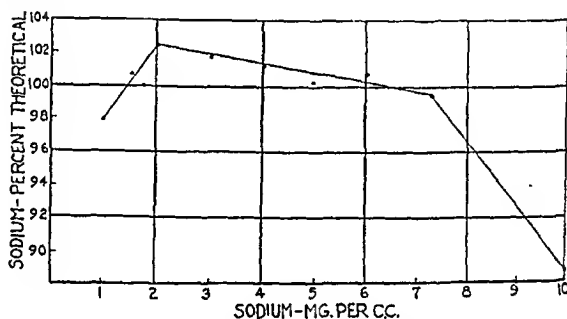


Fig. 1.—Relationship of concentration of sodium to the amount found.

Interference of Thorium.—In her article Strauss states that the ashes extracted with 0.1 per cent phosphoric acid are thorium free, but this has not been our experience. Such extracts contain considerable amounts of thorium, which is precipitated by the pyroantimonate reagent, and therefore give high results. Data bearing on this point are given in Table I.

TABLE I
INTERFERENCE OF THORIUM WITH SODIUM DETERMINATION

SAMPLE NUMBER	H ₂ PO ₄ USED FOR EXTRACTION (%)	SODIUM TAKEN* (MG.)	SODIUM FOUND (MG.)	BLANK VALUE
1	0.1	3.14	3.83	0.69
2	0.1	2.61	3.31	0.70
3	0.1	0.00	0.70	0.70
4	0.4	3.14	3.27	0.13
5	0.4	0.00	0.17	0.17

*Per cubic centimeter of ash solution.

Limits of the Method.—The work of Kramer and Gittleman indicated their method to be accurate within ± 2 per cent. Balint⁶ has claimed that the method gives results which are about 3 per cent higher than the theoretical, and Peters and Van Slyke⁷ suggest that the results be divided by a factor of 1.03. It should be noted that Eisenman, and Peters and Van Slyke, give the impression that the method is accurate only when the amount of sodium determined

is between 2 and 3.75 mg. per cubic centimeter of ash solution. Balint, on the other hand, gives the permissible range as 0.5 to 6 mg. We felt that this point was worthy of further study and have found that the range of 1.0 to 7.5 mg. gives a reasonable degree of accuracy (Fig. 1).

TABLE II
ANALYSIS OF URINE WITH ADDED SODIUM CHLORIDE

SAMPLE NUMBER	COMPOSITION OF SAMPLE (C.G.)		SODIUM FOUND TOTAL (MG.)	SODIUM ADDED (MG.)	SODIUM RECOVERED (MG.)	PER CENT RECOVERY
	URINE	0.8% NaCl				
1	5	0	18.80	0		
2	5	1	21.97	3.14	3.17	100.9
3	4	2	21.39	6.29	6.35	100.9
4	3	3	20.82	9.43	9.54	101.2
5	2	4	20.14	12.58	12.62	100.3
6	1	5	18.51	15.72	14.75	93.8*

*The results have invariably been low when 1 c.c. samples of urine (and, occasionally, 2 c.c. samples) were ashed, probably because of the bulky and gelatinous precipitates formed in the ash solutions. Apparently in these cases a smaller proportion of the thorium is converted to the oxide, hence more is left to form the phosphate. A similar situation was encountered in analyzing the synthetic urines described in Table III; this probably explains the low results obtained on these samples.

TABLE III
ANALYSIS OF MIXTURES OF KNOWN COMPOSITION

SUBSTANCE	SAMPLE A	SAMPLE B	SAMPLE C
NaCl	20 Gm.	10 Gm.	None
Other salts*	10.25 Gm.	10.25 Gm.	10.25 Gm.
Urea	20 Gm.	20 Gm.	20 Gm.
Water added	1,200 c.c.	1,200 c.c.	1,200 c.c.
Mg. sodium per c.c., theory	0.55	3.27	0.0
Mg. sodium per c.c., found	6.31	3.20	0.0

*The composition of this mixture was 6 Gm. of KH_2PO_4 , 1.1 Gm. of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.65 Gm. of MgSO_4 , and 2.5 Gm. of $(\text{NH}_4)_2\text{SO}_4$.

Accuracy of the Method as Applied to Urine.—This accuracy has been demonstrated in three ways: (1) recovery of sodium added to urine (Table II), (2) analysis of known mixtures approximating the composition of urine (Table III), and (3) demonstration of the agreement of the values obtained with those of the gravimetric method of Butler and Tuthill.⁸ It might be stated further that the method is remarkably precise, since it is not at all unusual to have all the pairs of five duplicate determinations agree within 0.02 mg. sodium (that is, about 0.7 per cent difference), and differences of as much as 0.05 mg. are very rare.

REFERENCES

1. Strauss, M. B.: The Use of Thorium Nitrate in the Rapid Ashing of Serum and Urine, *J. Biol. Chem.* 118: 331, 1937.
2. Stolte, K.: Eine einfache und zuverlässige Methodik der Aschenanalyse, *Biochem. Ztschr.* 35: 104, 1911.
3. Tisdall, F. F., and Kramer, B.: Methods for the Direct Quantitative Determination of Sodium, Potassium, Calcium, and Magnesium in Urine and Stools, *J. Biol. Chem.* 48: 1, 1921.
4. Kramer, B., and Gittleman, I.: An Iodometric Method for the Determination of Sodium in Small Amounts of Serum, *Ibid.* 62: 353, 1924.
5. Wakeman, A. M., Eisenman, A. J., and Peters, J. P.: A Study of Human Red Blood Cell Permeability, *Ibid.* 73: 567, 1927.
6. Bahat, M.: Eine jodometrische Mikrobestimmung des Natriums, *Biochem. Ztschr.* 150: 423, 1924.
7. Peters, J. P., and Van Slyke, D. D.: Quantitative Clinical Chemistry, Methods, Baltimore, 1932, Williams & Wilkins Co., pp. 737-740.

THE STANDARDIZATION OF pH MEASUREMENTS WITH A PRECISION GLASS ELECTRODE AT VARIOUS TEMPERATURES*

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INTRODUCTION

THE pH values of a number of biological products and bacterial media, obtained by colorimetric and electrometric methods, have been presented in some recent papers.^{1, 2} In several procedures employed, the test fluids were diluted with fixed quantities of distilled water or physiologic saline solution. In the present work the pH values of a number of buffer mixtures, diluted with varying quantities of distilled water and saline solution, have been determined between the temperatures of 10° C. and 38° C. Since the measurement of pH values is so fundamental in all biological laboratories, the use of a standard pH scale is essential. The pH scale adopted throughout this work is based upon the pH values of primary potassium phthalate. The pH values of the buffer mixtures studied here have been tested by employing these values in computing the dissociation constants of the corresponding buffer acids by means of an approximation of the Debye-Hückel limiting law.

Earlier work on the standardization of the pH scale has been fully described by Clark.³ Recent studies of Hitchcock,^{4, 5} MacInnes,⁶ and their associates have provided a fundamental basis for further pH studies. Our work is based essentially upon the methods employed by these workers.

The dissociation constant K' of a weak acid HA is a function of the pH value of a solution of the acid and its salt:

$$pK' = pH - \log \frac{C_A}{C_{HA}} \quad (1)$$

By replacing the stoichiometric concentrations C_A and C_{HA} by the activities of the acid and its negative ion constituent, pK' may be expressed as a function of the thermodynamic dissociation constant K :

$$pK' = pK + \log \frac{f_A}{f_{HA}} \quad (2)$$

For moderately dilute solutions Hitchcock has proposed an approximation of the limiting law:⁷

$$\log \frac{f_A}{f_{HA}} = B\mu - A \sqrt{\mu} \quad (3)$$

where A is the familiar Debye-Hückel constant and B is a constant independent of the concentration but may vary for different solutes. The preceding expres-

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sion for $\log \frac{f_A}{f_{HA}}$ is substituted into equation (2); pK' is obtained as a function of the thermodynamic dissociation constant and of an approximate form of the limiting law:

$$pK' = pK - A \sqrt{\mu} + B\mu \quad (4)$$

The following procedure was employed in our work. pK' values were first computed from the measured pH values of the buffer mixtures by means of equation (1). It was found that by employing the apparent linear relation between the expression $(pK' + A \sqrt{\mu})$ and the ionic strength μ , the constants pK and B in equation (4) could then be evaluated by the Method of Least Squares. pK' values for the buffer acids computed by means of equation (1) were then compared with pK' values computed by means of equation (4). The deviations between the two sets of pK' values for a given buffer acid at a given temperature serve as a measure of the degree of conformity of the pH scale adopted in this work with thermodynamic principles and an approximate form of the Debye-Hückel theory.

The foregoing method was essentially that of extrapolation employed by Bjerrum and Unmack⁸ to evaluate the thermodynamic dissociation constant of citric acid and other weak acids at various temperatures.

EXPERIMENTAL

The procedure adopted consisted in the measurement of pH values of buffer solutions set up in a galvanic cell with liquid junction:

Glass, Solution X:KCl (saturated): HgCl, Hg

An Electron Ray Meter was employed in conjunction with a MacInnes-Belcher durable condenser type glass electrode. The latter has been amply described in the literature.⁹⁻¹¹ The Electron Ray Meter is a potentiometer capable of recording either millivolts or pH units. The essential parts of the meter consist of a cathode-ray tube which in conjunction with a triple grid pentode detector and amplifier, operate as null-point indicator. In these determinations the instrument was set to record directly in pH units.

Buffer solutions were prepared with Baker's "analyzed" salts. A 0.05 molar solution of primary potassium phthalate ($KHC_8H_4O_4$) was employed as a primary pH standard. The latter was prepared from the crystalline salt purchased from the National Bureau of Standards. The pH values of this solution have been carefully determined by MacInnes and his associates.⁶ Using their work as a basis, the following pH values of 0.05 molar $KHC_8H_4O_4$ were adopted as standards of reference throughout this work:

t°C.	pH
10	4.00
25	4.00
38	4.02

Test solutions were prepared from the buffer mixtures by dilution with appropriate volumes of distilled water or 0.1454 molar sodium chloride solution.

pH measurements were carried out by enclosing the glass electrode filled with the test fluid in an electrically grounded metal case, which in turn was

TABLE I
PHOSPHATE BUFFERS

(1)	(2)	(3)	(4)	(5)				(6)	(7)	(8)	
% DILUTION	KH_2PO_4	Na_2HPO_4	μ	pk'		38° C.		NaCl	μ (1)	25° C.	38° C.
				25° C.		38° C.					
				OBSERVED	CALCULATED*	OBSERVED	CALCULATED†				
				OBSERVED	CALCULATED*	OBSERVED	CALCULATED†				
0	0.02500	0.02500	0.10000	6.86	6.85	6.83	6.83				
10	0.02250	0.02250	0.09000	6.86	6.87	6.83	6.85	0.01454	0.10454	6.84	6.82
25	0.01875	0.01875	0.07500	6.89	6.88	6.86	6.86	0.03635	0.11135	6.82	6.80
50	0.01250	0.01250	0.05000	6.93	6.93	6.90	6.90	0.07271	0.12271	6.78	6.76
75	0.00625	0.00625	0.02500	7.00	6.99	6.97	6.97	0.10906	0.13406	6.75	6.73
90	0.00250	0.00250	0.01000	7.06	7.06	7.03	7.03	0.13087	0.14087	6.72	6.70

*25° C. pK' = $7.20 - 1.518 \sqrt{\mu} + 1.34\mu$.†38° C. pK' = $7.18 - 1.557 \sqrt{\mu} + 1.42\mu$.TABLE II
BORAX BUFFERS

(1)	(2)	(3)	(4)						(5)	(6)	(7)	
% DILUTION	Na ₂ B ₄ O ₇	μ	pK'						NaCl	μ ₁₁	pK''	
			10° C.		25° C.		38° C.				25° C.	38° C.
			OBSERVED	CALCULATED*	OBSERVED	CALCULATED†	OBSERVED	CALCULATED‡				
0	0.05000	0.10000	9.30	9.30	9.18	9.18	9.05	9.05	0.01454	0.10454	9.15	9.03
10	0.04500	0.09000	9.30	9.30	9.18	9.18	9.05	9.05	0.03635	0.11135	9.14	9.00
25	0.03750	0.07500	9.29	9.29	9.18	9.18	9.05	9.06	0.07271	0.12271	9.11	8.96
50	0.02500	0.05000	9.29	9.30	9.18	9.19	9.05	9.06	0.10906	0.13406	9.08	8.92
75	0.01250	0.02500	9.30	9.30	9.19	9.19	9.08	9.08	0.13087	0.14087	9.06	8.90
90	0.00500	0.01000			9.21	9.21	9.09	9.10				

*10° C. pK' = $9.35 - 0.493 \sqrt{\mu} + 1.10\mu$.†25° C. pK' = $9.25 - 0.506 \sqrt{\mu} + 0.33\mu$.‡38° C. pK' = $9.11 - 0.519 \sqrt{\mu} + 0.71\mu$.

enclosed in an incubator with thermostatic temperature control. By means of the latter the temperature around the glass electrode assembly (glass and calomel electrodes) was kept constant within $\pm 0.5^\circ \text{C}$. during any measurement. When not in use, the glass electrode was filled with distilled water. By this practice the residual potential within the glass membrane (asymmetry potential) is maintained at a low constant level.¹⁰

EXPERIMENTAL RESULTS

The pH values of the unknown test solutions in the accompanying tables represent the results of at least three concordant measurements at each temperature. For the test solutions between pH 4.0 and 9.0, the MacInnes-Belcher glass electrode in conjunction with the Electron Ray Meter, functioned accurately, well within the limits ± 0.02 pH unit. For test solutions having values ranging from pH 9.0 to 9.3, the accuracy of the instrument was reduced to about ± 0.03 pH unit. Employing the pH values assigned to the standard phthalate buffer, the apparatus yielded pH values for the undiluted buffers which differed from the values obtained by Hitchcock and MacInnes with the hydrogen electrode by ± 0.01 pH unit. However, the pH values of the acetate buffer mixture at 25°C . and that of the borate buffer at 38°C . differed from the values obtained by MacInnes and Hitchcock by ± 0.02 pH unit.⁴⁻⁶

The following values of the Debye-Hückel constant A^{12} were employed in equation (4):

$t^\circ \text{C}$.	A
10	0.493
25	0.506
38	0.519

The value of A at 10°C . was obtained by graphical interpolation. Between 0°C . and 18°C ., A is approximately a linear function of the temperature.

Table I summarizes the results obtained with the phosphate buffer mixtures at 25° and 38°C . Since these solutions consist of 1:1 buffer mixtures, C_A and C_{NA} are equal and the pK' values computed from equation (1) are numerically equal to the measured pH values of the buffers. Likewise pK'' values are equal to the measured pH values of the saline buffer mixtures. The pH values have, therefore, been omitted from this table.

Column (1) gives the dilution factor or the percentage dilution with distilled water or 0.1454 molar sodium chloride solution. Columns (2) and (3) give the molar concentrations of primary potassium phosphate (KH_2PO_4) and secondary sodium phosphate (Na_2HPO_4), respectively. Column (4) gives the values of the ionic strength of the test solutions. Column (5) gives the observed and calculated pK' values of the buffer acids by means of equation (1) and equation (4), respectively. Columns (6), (7), and (8) contain data for the buffer solutions diluted with 0.1454 molar sodium chloride solution, which have been included in the table for comparison with the corresponding data in aqueous solution (Table I).

For the phosphate buffer mixtures equation (4) was altered in accordance with the interionic attraction theory by the use of $3A$ in place of A . The ionic strength of the aqueous buffer solutions was taken as four times the con-

TABLE III
SODIUM ACETATE—ACETIC ACID BUFFERS*

(1)	(2)	(3)	(4)	(5)			(6)			(7)	(8)	(9)		(10)	
% DILUTION	CH ₃ COOH	CH ₃ COONa	μ	10° C.	25° C.	38° C.	10° C.	25° C.	38° C.	NaCl	μ (1)	25° C.	38° C.	25° C.	38° C.
0	0.05230	0.05000	0.05000	4.66	4.68	4.66	4.68	4.70	4.68						
10	0.04707	0.04500	0.04500	4.66	4.68	4.66	4.68	4.70	4.68	0.01454	0.03956	4.68	4.66	4.70	4.68
25	0.03923	0.03750	0.03750	4.67	4.69	4.67	4.69	4.71	4.69	0.03625	0.07387	4.65	4.63	4.67	4.65
50	0.02615	0.02500	0.02500	4.67	4.70	4.68	4.69	4.72	4.70	0.07271	0.09773	4.65	4.63	4.67	4.65
75	0.01308	0.01250	0.01250	4.70	4.72	4.70	4.72	4.74	4.72	0.10906	0.12158	4.64	4.62	4.66	4.63
90	0.00523	0.00500	0.00500		4.76	4.74		4.78	4.76	0.13087	0.13589	4.64	4.62	4.65	4.63

*To compute pK' from equation (4):

$$10^{\circ} \text{ C. } pK' = 4.76 - 0.493 \sqrt{\mu} + 0.61\mu.$$

$$25^{\circ} \text{ C. } pK' = 4.78 - 0.506 \sqrt{\mu} + 0.87\mu.$$

$$38^{\circ} \text{ C. } pK' = 4.76 - 0.519 \sqrt{\mu} + 0.52\mu.$$

centration of either phosphate salt. The ionic strength of the saline buffer solutions was taken as the molar concentration of sodium chloride and four times the molar concentration of either phosphate salt. The average differences between pK' values obtained from equation (1) and those computed from equation (4) are ± 0.01 and ± 0.00 at 25°C . and 38°C ., respectively.

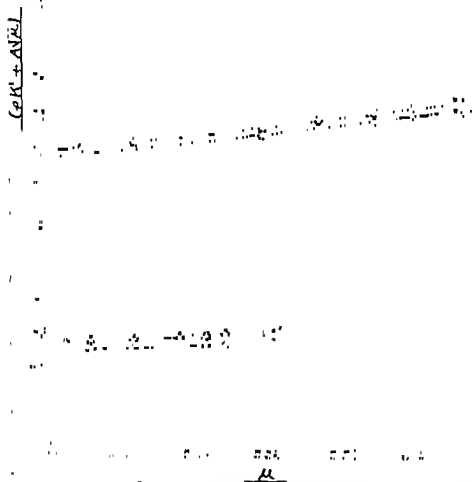
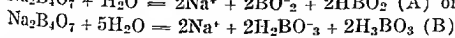
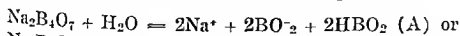


Fig. 1.—Extrapolation of pK' data for acetate buffers, triangles; phosphate buffers, circles; and borax buffers, squares.

The data for the borax buffers are summarized in Table II. The following possible paths of the hydrolytic reactions have been proposed:⁴



Hitchcock has shown that neither reaction (A) nor reaction (B) go to completion, as indicated by an actual alkalinity of 2×10^{-5} mols per liter of hydroxyl ions in the borax solutions. He suggests that this may be due to a reaction between the borate ion and water to form undissociated acid. Therefore, C_{HA} was obtained by adding C_{OH} (2×10^{-5} mols per liter) to the molar concentration of sodium borate. Likewise, C_A was taken as equal to the difference between the molar concentration of borax and the hydroxyl-ion concentration.

Since pK' values computed by means of equation (1) were numerically equal to the observed pH values, the latter have been omitted from the table (Table II).

For the aqueous borax solutions the ionic strength was taken as twice the molar concentration of sodium borate. For the borax buffers diluted with sodium chloride solution, the ionic strength was taken as the sum of the molar concentration of sodium chloride and twice the molar concentration of sodium borate. The average differences between pK' values obtained from equation (1) and those computed by means of equation (4) are ± 0.00 , ± 0.00 , and -0.01 at 10°C ., and 25°C ., and 38°C ., respectively.

Table III summarizes the data for the acetate buffer mixtures. Both the ionic strength of the aqueous buffer mixtures and the concentration of the negative ion constituent, C_A , were taken as equal to the molar concentration of sodium acetate. The concentration of the undissociated acid, C_{HA} , was taken as equal to the molar concentration of acetic acid in the buffer mixtures. The ionic strength of the saline buffers was taken as the sum of the molar concentrations of sodium chloride and sodium acetate.

The average differences between pK' values obtained from equation (1) and those computed by means of equation (4) were $+0.01$ at 10°C ., 25°C ., and 38°C ., respectively, for the acetate buffers.

Fig. 1 shows graphically the method of extrapolation employed to evaluate the constants pK and B in equation (4). The points on the lines were obtained by the Method of Least Squares, using the appropriate data in Tables I, II, and III for aqueous solutions of the phosphate, borate, and acetate buffer mixtures. The best straight lines were then drawn through these points (Fig. 1). The intercepts on the $pK' + A\sqrt{\mu}$ axis give the pK values of the buffer acids at infinite dilution. A comparison of limiting pK values obtained in this study with those obtained by others is given in Table IV.

TABLE IV

LIMITING pK VALUES OF SOME WEAK ACIDS AT 10°C ., 25°C ., AND 38°C .

ACID	$t^\circ \text{C}$.	(6)*	(5)†	(13)	(14)	(15)	(16)
CH_3COOH	10	4.76		4.762			
	25	4.78		4.7572	4.7562		
	38	4.76	4.766				
H_2BO_3	10	9.35				9.389	
	25	9.25				9.237	
	38	9.14	9.143				
H_3PO_4 (pK_2)	25	7.20					7.206
	38	7.18	7.19				

*Obtained by the method of least squares from the appropriate data in the present work.

†Interpolation of data of Harned and Ehlers,¹¹ Owen,¹² and Nims,¹³ for 38°C by Hitchcock and associates.

Table V gives the pH values at various temperatures of standard buffers commonly employed in electrometric pH measurements. These are compared with the pH values obtained by Hitchcock,^{4, 5} MacInnes,⁶ and their associates at corresponding temperatures, using the hydrogen electrode. In no case do the values obtained in the present work differ from the values obtained by these workers by more than $+0.02$ pH unit.

TABLE V

COMPARISON OF pH VALUES OF STANDARD BUFFER SOLUTIONS AT VARIOUS TEMPERATURES

BUFFER MIXTURE	t° C.	(G)	(6)	(4) (5)
0.05 M $\text{KH}_2\text{H}_2\text{O}_4$	10	4.00		
	12		4.000	
	25	4.00	4.000	4.010
	38	4.02	4.015	4.025
0.01 M $\text{CH}_3\text{COOH} + 0.01 \text{ M CH}_3\text{COONa}$	10	4.70*		
	12		4.710	
	25	4.72*	4.700	4.714
	38	4.70*	4.710	
0.025 M $\text{KH}_2\text{PO}_4 + 0.025 \text{ M Na}_2\text{HPO}_4$	25	6.86		6.855
	38	6.83		6.835
0.05 M $\text{Na}_2\text{B}_4\text{O}_7$	10	9.30		
	25	9.18		9.180
	38	9.05		9.070

(G) Measured pH values obtained in the present work.

*Actual concentrations of sodium acetate and acetic acid used here: 0.01308 M CH_3COOH and 0.01250 M CH_3COONa .

DISCUSSION

Examination of the pH data in the tables shows that the pH values of all the buffer solutions increase with increasing aqueous dilution. While the increases are relatively small for the acetate and borate buffer mixtures, the phosphate buffer suffered a large increase in pH, amounting to 0.2 pH unit at 90 per cent dilution with distilled water. On the other hand, all the buffer mixtures suffered a marked decrease in pH, with increasing dilution with sodium chloride solution. These results are in agreement with those obtained by other workers.^{8, 17} Moreover, while increases in temperature affected the undiluted acetate and phosphate buffers least, the borax buffer suffered a decrease of 0.25 pH unit between 10° and 38° C. It has already been pointed out that the pH values of the undiluted buffers showed good agreement with the values obtained by Hitehoeek and MacInnes with the hydrogen electrode.

Since it is possible to measure mean ionic activities only, pH as a function of the negative logarithm of the hydrogen-ion activity cannot be placed on a strict theoretical basis. With this fact in mind, any attempt to provide a strict thermodynamic basis for the pH scale is necessarily limited. The efforts of MacInnes and his associates in this direction are noteworthy. Employing extremely dilute buffers, they applied the limiting law to pK data on acetate buffer mixtures at 12° C., 25° C., and 38° C. However, values of the Debye-Hückel constant A were obtained which were greater than those required by theory.⁶ They indicate that if pH were a function of mean ionic activities, extrapolation of pK data would yield curves whose limiting tangents would approach the values of A obtained with their data on acetate buffer mixtures.^{18, 19}

Since the solutions employed in this work were not sufficiently dilute to warrant the application of the limiting law, an approximation of the Debye-Hückel theory was used. The method of extrapolation applied here gave limiting pK values for the buffer acids which showed fair agreement with the values obtained by other workers, with the exception of boric acid at 10° C. The pK value of boric acid, obtained by the procedure described previously, was about 0.04 unit lower than that obtained by Owen for the same temperature. The values of B in equation (4) varied with the temperature and the type of buffer acid.

SUMMARY

Using the pH values assigned to a standard phthalate buffer as reference, the pH values of acetate, phosphate, and borate buffers diluted with water and with sodium chloride solution have been determined at 10° C., 25° C., and 35° C. with a glass electrode in a cell with liquid junction. pK' and pK'' values have been computed from the appropriate pH data for the aqueous and saline buffer solutions, respectively. Employing the apparent linear relation between $(pK' + A\sqrt{\mu})$ and μ , limiting pK values of the buffer acids have been computed. Tests have been made of the degree of conformity of the pH scale adopted in this work with the limiting pK values and an approximation of the Debye-Hückel limiting law. Within the limits of experimental error the MacInnes-Belcher glass electrode, in conjunction with the Electron Ray Meter, gave accurate and reproducible pH values which were in agreement with the values obtained by others with the hydrogen electrode.

REFERENCES

1. Glaubiger, A., and Falk, K. G.: The Determination of pH Values of Biologic Fluids. I, *J. LAB. & CLIN. MED.* 25: 369, 1943.
2. Glaubiger, A.: The Determination of pH Values of Biological Fluids. II. The Influence of Temperature, Dilution, and the Type of Diluent Upon the pH Values of Bacteriologic Media, *J. LAB. & CLIN. MED.* 25: 1311, 1940.
3. Clark, W. M.: The Determination of Hydrogen Ions, ed. 3, Baltimore, 1928, Williams & Wilkins Co.
4. Hitchcock, D. I., and Taylor, A. C.: The Standardization of Hydrogen Ion Determinations. I. Hydrogen Electrode Measurements With Liquid Junction, *J. Am. Chem. Soc.* 60: 1812, 1937.
5. Hitchcock, D. I., and Taylor, A. C.: The Standardization of Hydrogen Ion Determinations. II. A Standardization of the pH Scale at 35°, *J. Am. Chem. Soc.* 60: 2710, 1938.
6. MacInnes, D. A., Belcher, D., and Shedlovsky, T.: The Meaning and Standardization of the pH Scale, *J. Am. Chem. Soc.* 60: 1394, 1938.
7. Hitchcock, D. I.: The Extrapolation of Electromotive Force Measurements to Unit Ionic Activity, *J. Am. Chem. Soc.* 50: 2076, 1928.
8. Bjerrum, N., and Unmack, A.: Elektrometrische Messungen mit Wasserstoffelektroden in Mischungen von Säuren und Basen mit Salzen, *Kgl. Danske Videnskab. Selskab. Math.-fys. Medd.* 9: 1, 1929.
9. MacInnes, D. A., and Belcher, D.: Further Studies on the Glass Electrode, *J. Am. Chem. Soc.* 53: 3315, 1931.
10. MacInnes, D. A., and Belcher, D.: A Durable Glass Electrode, *Indust. & Engin. Chem. Anal. Ed.* 5: 9, 1933.
11. MacInnes, D. A., and Longworth, L. G.: The Measurement and Regulation of pH With the Glass Electrode, *Tr. Electrochem. Soc.* 71: 73, 1937.
12. MacInnes, D. A.: Principles of Electrochemistry, New York, 1939, Reinhold Publishing Co.
13. Harned, H. S., and Ehlers, J. W.: The Dissociation Constant of Acetic Acid From 0° to 35°, *J. Am. Chem. Soc.* 54: 1350, 1932.
14. MacInnes, D. A., and Shedlovsky, T.: The Determination of the Ionization Constant of Acetic Acid at 25° From Conductance Measurements, *J. Am. Chem. Soc.* 54: 1429, 1932.
15. Owen, B. B.: The Dissociation Constant of Boric Acid From 10° to 50°, *J. Am. Chem. Soc.* 56: 1695, 1934.
16. Nimis, L. F.: The Second Dissociation Constant of Phosphoric Acid From 20° to 50°, *J. Am. Chem. Soc.* 55: 1946, 1933.
17. Kolthoff, I. M., and Bosch, W.: The Influence of Neutral Salts on Acid-Salt Equilibria. I. A Contribution to the Knowledge of the Standard Value Used for Calculating the Activity Exponent pH of the Hydrogen Ion Concentration, *Rec. Trav. Chim.* 46: 430, 1927.
18. Brown, A. S., and MacInnes, D.: The Determination of Activity Coefficients From the Potentials of Concentration Cells With Transference, *J. Am. Chem. Soc.* 57: 1256, 1935.
19. Shedlovsky, T., and MacInnes, D. A.: The Determination of Activity Coefficients From the Potentials of Concentration Cells With Transference, *J. Am. Chem. Soc.* 55: 503, 1937.

MEDICAL ILLUSTRATION

MOULAGE PROSTHESIS

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WITH ILLUSTRATIONS BY JOHN T. STRINGER, JR., AND CARL DAME CLARKE

THE art of face and body restoration is not new. In fact, the Hindus developed methods for the repair of nasal defects which today are considered good operative procedure in rhinoplasty. This was only one of their difficult plastic feats. According to Kazanjian, Rowe, and Young,¹ the literature of plastic surgery dates back as far as Celsus (25 B.C.) and Galen (A.D. 131-201). They call attention to Paul of Aegina (A.D. 630) and Branca (1442). They also mention Ambroise Paré (1541), Tagliacozzi (1545), Pierre Fauchard (1728), Bourdet Suerson and Delabarre (1820), Hullivan, Kingsley, Claude Martin, Nelaton, and Zeis (1900), and others. In 1875 Martin was the first to conceive of immediate prosthesis.

From the prewar plastic surgeons, Morestin, Lexer, and others, it will be noted that the medical and dental literature contains many articles, methods, and case reports concerning surgical and mechanical means of correcting pathologic deformities of the face. The horrible maiming effects of the first World War produced innumerable patients for surgeons, dentists, and moulage workers to treat. It was during war times that the art and science of prosthesis took gigantic strides forward.

In consideration of the fact that another World War is now in progress, it is obvious that there will be hundreds, if not thousands, of cases demanding prosthetic appliances.

In 1938 I published a book on the subject of *Molding and Casting*² in which I discussed previous work in moulage prosthesis in detail, as well as methods in present-day use for producing these prostheses. Since that time there have appeared on the market many synthetic plastics which lend themselves readily to the making of prostheses. It is my desire to describe herein the methods for making both a moulage prosthesis on what would ordinarily be a very simple case and a prosthesis for a more advanced case. Such work definitely falls on the scientific illustrator as well as the physician. The formulas for prosthetic work may be compounded by the worker, or the compositions may be purchased already prepared.

The physician can make a single mold and cast of the part to be reconstructed if the case is a simple one. From this mold he can make a reconstruction in wax of the missing part. Practically any dental laboratory can repro-

duce a synthetic plastic duplicate from this wax original. False teeth are made in the same manner by the dental laboratory from a mold supplied by the dentist.

Above all else the case in mind for reconstructive procedure should be considered carefully before any work is begun. The worker should determine whether it is more suitable for treatment by plastic surgery or by reconstructive prosthesis. It should be clearly understood that while the result of surgery may be more satisfactory, its application may involve danger and pain that is distributed over a period of weeks, with uncertain and often mortifying results. The prosthesis, while not a permanent cure, is without danger and involves no pain to the patient either in the making or in the wearing. For patients unable to withstand the debilitating effects of operative procedure, a prosthetic appliance is strongly recommended. I do not advocate the use of a prosthesis in preference to plastic surgery for the correction of facial defects. The case in question must be the deciding factor. However, it cannot be stressed too forcefully that plastic surgery alone should not be considered in every case.

Lederer³ writes that skin cannot be molded in the manner of clay; hence it is very difficult to produce a lifelike nose or ear by plastic surgery. The nose is constantly under the scrutiny of others, and it stands to reason that the patient would be no worse off without his nose or perhaps with a small adhesive dressing than he would with a few clumps of grafted skin, which at best would still be extremely conspicuous. Lederer maintains further that he, as well as other plastic surgeons, has been disappointed in the net result of his painstaking labors and, in a measure, feels guilty for having subjected his patients to operations which not only brought them great economic loss and suffering, but in many instances jeopardized their health and lives. They had scars remaining where tissue was grafted, spent many uncomfortable weeks in a cast (when Italian grafts were utilized), lost time from their work and in some instances had to accept primary failure owing to poor healing.

Van Dijk⁴ found that the ears he formed by plastic surgery were too bulky and not sufficiently erected, and that the fossa scaphoidea and the anthelix were not built up. He planned in his next patient to get the ear more erect through implants of cartilage, thus giving it a more shell-like appearance.

More and more the surgeon is beginning to recognize a substitute in the form of a prosthesis for certain plastic procedures. These two phases of the healing art are inseparably related. The future of restorative work, just as in the field of prevention, lies in the closest cooperation between physician and surgeon and the prosthetist.

Furthermore, if the surgeon is working with a prosthetist, the necessity of adequate surgical preparation for the application of an artificial restoration cannot be too strongly stressed. The failure of many prosthetic attempts is often caused by the unfortunate necessity of adapting restorative to areas of soft tissue which have been distorted and contracted by unnecessary

scar tissue and improper postoperative procedure. The success of a prosthetic restoration is in a great measure increased as the degree of mutilation is lessened.

The ability of the surgeon and of the moulage worker has much to do with the final result in either case. I am unable to criticize surgical technique but I am more familiar with the procedure of moulage making and the final prosthetic appliance. In my opinion these prostheses suffer from the same defects that are prevalent in most medical art work. In short, they lack artistic skill in execution and naturalness in its simplest form. The artificial appliance to the human face should, above all else, look realistically natural and individually suitable. The conspicuous errors of most poor prostheses are generally the result of a lack of artistic ability and appreciation on the part of the surgeon or prosthetist. These difficulties may be overcome by having the prosthetic appliance made by a skilled prosthetist in close cooperation with the plastic surgeon. Because the surgeon or dentist has considerable ability in surgery or moulage making does not necessarily mean that he is an artist. His ability or judgment to choose the correct feature in size and relative proportions to be fitted next to other adjoining features of flesh and blood may be lacking.

Another eventual stumbling block to the surgeon and dentist is the final coloring of the artificial part. It takes considerable experience in handling pigments and a natural color sense to blend perfectly the many different hues into the prosthesis to match the surrounding tissue. Hair, bristle brushes and airbrushes, pigments and pastes are often used for this purpose. The artist possessing this color sense yearns to "touch up" the average monochromed prosthesis which in its single hue is not natural. He desires further to eliminate the many false tones and shades of a modernistic pictorial nightmare. For the making of the best facial prosthesis close cooperation between the physician, dentist, and prosthetist is essential. Even the physician having little or no artistic ability can make acceptable prostheses of simple cases, provided he works in close cooperation with a dental laboratory.

In a simple case of prosthesis the first step is to obtain a mold or impression of the defective part. An agar composition is generally used for such work. This molding material may be purchased under the trade name of "Hydrocolloid" (Detroit Dental Mfg. Co.), "Dentocoll" (L. D. Canlk Co.), and others. A serviceable composition may be prepared from one of the formulas published in the articles listed in the references. If the worker is interested only in the results he obtains rather than in the materials he uses, he will find it more desirable to purchase the composition already prepared. The commercial preparation comes in a handy collapsible tube which is placed in boiling water for ten minutes. It is then transferred to lukewarm water and allowed to cool to a point where the tube does not feel uncomfortable when placed on the bare wrist. An agar composition should never be applied when it is so warm that it would be uncomfortable to the patient. This material should not set until it reaches a point ($+2^{\circ}$ C.) slightly above body temperature (37.5° C.). Even though the patient can withstand the heat, the material must



Fig. 1.—A. Front view of a defective area on the nose.
 B. A side view of the same area.
 C. The warm agar composition being applied to the nose directly from the tube.
 D. A front view of the agar mold in place just after setting.
 E. The agar mold being removed.
 F. The agar mold being filled with plaster of Paris, which sets to form the positive.

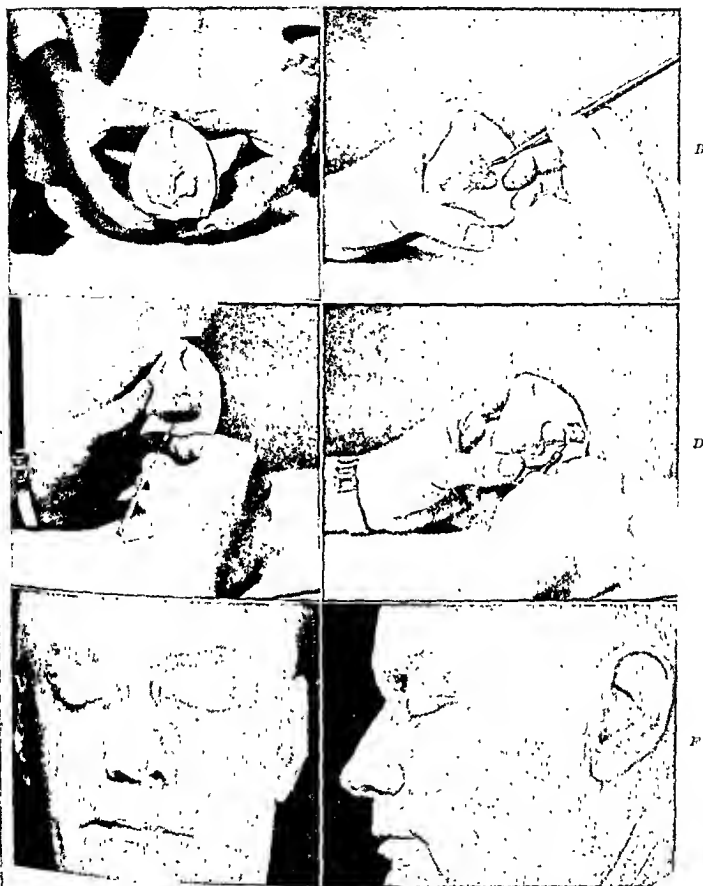


Fig. 2.—A The plaster impression after removal from the agar mold. The agar mold can be seen to the left. Part of the defect has been filled with additional plaster to allow a space for breathing. This is done with a soft brush and a plaster-water mixture.

B. The remainder of the defective area is filled with a wax composition (the formulas for which are in the text). A brush is used for applying the hot wax mixture. This is continued until the area is filled with the wax.

C. The wax is then carved down with an ordinary scalpel and shaped to follow the natural contours of the ala of the nose. A sharp, pointed probe is inserted into the wax from the inner side and the wax is removed. This wax will not adhere strongly to the wet plaster.

D. Showing the wax prosthesis being removed from the plaster nose.

E. The wax is then fitted into the patient's nose. If it fits perfectly it is turned over to a dental laboratory for reproducing into one of the modern synthetic plastics. A plastic is chosen to match the skin of the patient. This completed prosthesis is held in place by a solution of gum mastic dissolved in equal parts of alcohol and ether.

F. A side view of the patient with the prosthesis in place. E and F show the patient without any cosmetics over the prosthesis. The false area may be made to blend more perfectly with the skin by the use of "Covermark" or a similar cosmetic.

remain in place longer for sufficient cooling or setting time. When the tube has sufficiently cooled, its end is cut with a seissors and the material is squeezed over the afflicted part (Fig. 1c). In ten minutes the agar composition sets, after which it is removed (Fig. 1e). This mold should not be removed until it definitely feels firm. An entire ear can be cast in the same manner in a one-piece mold. This mold can then be filled with either hot wax or plaster of Paris. Wax should be used in making an ear as an intermediate step for a final rubber or synthetic plastic prosthesis. However, in considering the patient in the illustration (Fig. 1), it is best that the nose in this case be made in plaster of Paris, for the defective part must be modeled in wax on the plaster. This is done because the wax will separate readily from the wet plaster.

After the plaster nose has set, it is removed from the agar mold (Fig. 2a). Additional plaster is applied with a soft brush to the area represented by the opening in a normal nose for breathing. This will create the opening for breathing in the prosthesis. A brush is then dipped in a hot wax composition and applied directly over the defective area (Fig. 2b).

A wax composition suitable for this purpose is as follows:

	<i>Parts by weight</i>
Paraffin (62° C.)	10
Rosin	8
Carnauba wax	1

A ready-made wax may be purchased from dental supply stores. After the wax has set, it is carved with a pocketknife, scalpel, dental or sculptor's tool until it takes the shape of a normal nose (Fig. 2c).

A sharp, pointed probe is then inserted from the inner surface of the wax ala and with a slight pressure it should separate from the wet plaster (Fig. 2d). This wax piece can be tested on the patient (Figs. 2e and f). If it fits perfectly it is turned over to a dental laboratory to be made into one of the synthetic plastics, such as "Vernonite," "Crystalex," and "Luectone." Of course, shade should be chosen which matches the patient's skin as nearly as possible. If in the wax stage the prosthesis does not fit, it can be adjusted or changed so that it will fit the patient.

It is not advisable for the average physician or surgeon to attempt to do the final stage in the making of a prosthesis, since the dental laboratory is more fully equipped for such work and its charges are reasonable. After the synthetic plastic part is finished, the edge that comes in contact with the skin is painted with a solution of gum mastic dissolved in equal parts of alcohol and ether. The prosthesis is then put in place and held there with a finger until the solvent has evaporated and caused the gum mastic to hold it in place. This takes less than a minute in most instances.

A more complicated case (Figs. 3, 4, and 5) requires a greater knowledge in making prosthetic appliances. This case was a patient of the late Dr. A. Russell, whom I assisted in developing the prosthesis.

The first procedure was to obtain a plaster cast of the face in its original condition. Tubes were inserted in the nasal cavity and gauze was placed around

the tubes. The mold was then made of agar and reinforced with a plaster shell or mother mold (Fig. 3c-f). From this mold the plaster positive or cast which showed the complete facial defect was made. In this case ordinary gray plasteline was used to model a nose on the plaster ease (Fig. 4b). This, of course, requires artistic ability or training. However, the modeling of a nose is not essential, for a mold may be made of a living person who has a well-formed, if not an idealistic, nose. This person must be of the same type and general proportions as the patient. The cast from this second mold may be trimmed to fit into the open space on the plaster mask of the patient. The edges between the two casts may then be smoothed out by the use of plasteline.

From the modeled nose a mold was made of the reconstruction only (Fig. 4c) and a red wax impression was made from the mold (Fig. 4 c-g). The red wax cast is later removed from a piece mold; it sometimes requires boiling water to melt it. The subject of agar and wax compositions has been discussed.

Upon its removal from the mold (Fig. 4c), the back of the cast was modeled or carved into the shape (Fig. 4d). An extra knob was added to the wax impression as a support for attachment to the artificial palate which had been made previously.

After the wax impression was modeled or carved to the desired shape to suit the mechanical necessities of the cast, a piece mold of the entire cast, including front and back, was made (Fig. 4e). From this mold other red wax casts seen in *d* could be made. This is the same piece mold that is to be used to cast the final impression, provided the red wax cast fits the patient. Tests to determine the snugness of the fit were made on the original plaster cast of the face after the plasteline nose had been removed. Such tests were also made by putting the wax impression on the patient (Fig. 4f and g). The final impression was then cast.

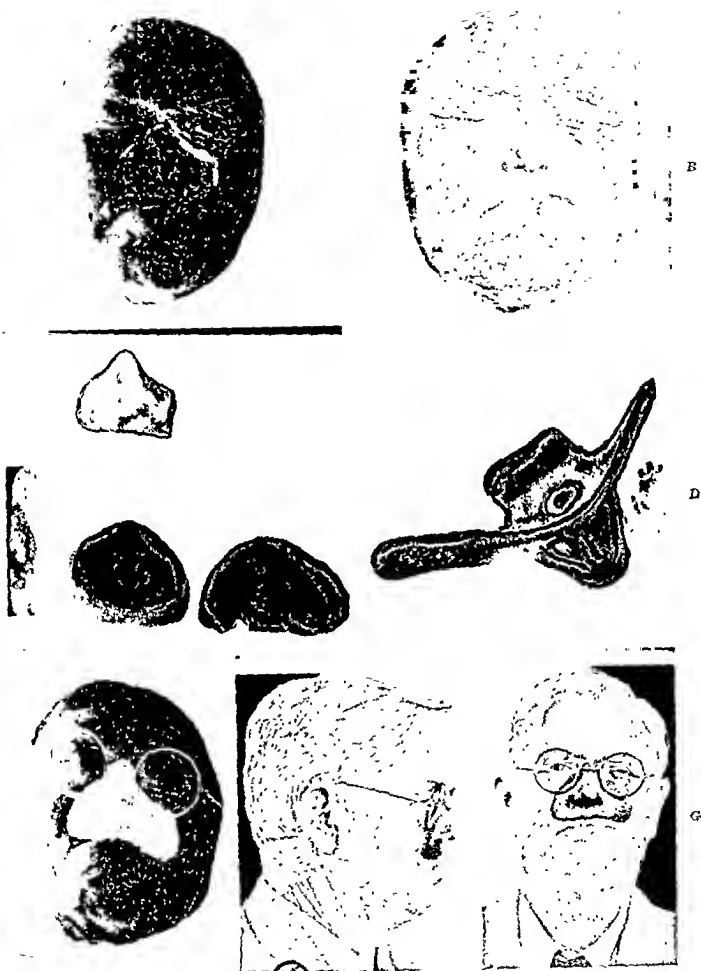
The material used for this final cast should be considered seriously. Numerous substances such as gelatin (Henning's method, described by Salamon; also elaborated on by Zinsser, Pont, Bereowitsch;⁵ Kazanjian, Rowe, and Young¹) and light metals, such as aluminum, have been used. Plastics, such as vulcanite or vulcanized rubber (not vulcanized latex) and celluloid compositions have been employed as positive casting materials.

The materials used in the past have been discussed fully in the articles listed as references. In my opinion the best materials for making a prosthesis to be worn on the face are the synthetic plastics previously discussed, and vulcanized latex. In comparison with rubber the synthetic plastics have one serious drawback when they are considered for large prostheses as, for example, an entire ear; they are hard and rigid, and somewhat heavier than rubber.

In making a moulage prosthesis of rubber, vulcanized latex, powdered fillers, and coloring agents are used. First, the powder is added to the flesh-colored latex until the desired consistency is obtained. Permanent water-soluble aniline dyes having a neutral or alkaline reaction are used for coloring the vulcanized latex to a flesh tint. The latex is then poured into a plaster mold and allowed to remain until the plaster absorbs enough water from the vulcanized latex to



Fig. 3.—A. Front view of a patient showing the healed lesion of a carcinoma. An artificial hard palate has been made and put in place.
 B. Side view of the same patient.
 C. Rubber tubes are inserted into the opening to facilitate breathing. Sterile gauze is packed around these tubes. Agar is used to make the mold. Some of this material was used in the illustration to cover the gauze.
 D. The agar mold near completion.
 E. The agar mold is reinforced with plaster.
 F. The removal of the two molds by gravity. The gauze that was used to fill the cavity in the face can be seen.



F

- Fig. 4.—A. The plaster impression of the face from the agar mold.
 B. A nose is modeled in plasteline on the plaster cast. A plaster impression is made of the plasteline model, from which a red wax nose is cast.
 C. While the red wax is still in place the back of the red wax is molded to form a squeeze piece mold.
 D. The underside of the red wax nose.
 E. The wax nose is tested with the glasses on the plaster cast.
 F. It is then fitted to the patient.
 G. Front view of the patient with the red wax nose in place.

cause a deposit of sufficient thickness. The surplus is then poured out. The latex can also be painted, layer by layer, into an agar, wax, or metal impression of a duplicate of the part to be replaced. Each layer of the rubber composition sets within a few minutes, after which another layer is applied. A celluloid solution may be poured or painted over this rubber to give it additional support and serve in a manner similar to the cartilage in the ear. The liquid celluloid may be made by dissolving scrap celluloid in butyl acetate. After the celluloid has set, more rubber may be painted over it, thus increasing this inflammable material in rubber. In other words, the rubber takes the place of flesh and the celluloid takes the place of cartilage. After drying, the resulting rubber cast is removed from the mold. Gentle heat speeds the setting time of such rubber casts.

It is far more difficult to trim and patch rubber impressions than to do the same work on wax or plaster impressions. Such trimming as is necessary must be done with very sharp wet knives. A wet blade cuts rubber more easily than a dry blade. Because of the difficulty of patching rubber positives, as many corrections as possible should be made on the mold. Another method is to obtain a thoroughly trimmed and patched wax cast of the individual, and make a second mold over this impression in any metal, except copper or zinc, by electrodeposition of the metal. Nickel may be electroplated on the wax, then copper, and then nickel again, thus increasing the copper between two layers of nickel. The metal mold is then used for casting rubber impressions. In most cases an unlimited number of positives may be obtained from a single mold.

In making a prosthesis of a hand, agar composition can be used to secure a one-piece mold. Wax is poured into the mold to obtain a wax positive. A one-piece plaster mold is made over the wax, and the wax is removed from the plaster with boiling water. Vulcanized latex is poured into the one-piece dry plaster mold to obtain a rubber hand. By this method even the pores of the skin are duplicated.

Vulcanized latex, which is sold under the trade name of "Vultex,"* is undoubtedly one of the best materials to use for making large artificial parts to be worn on the human body. It has many advantages: (1) Vulcanizing agents are unnecessary. (2) It sets in a closed plaster mold into which it may be poured through a funnel-like opening. (3) A separating medium is not necessary, as vulcanized latex does not stick to plaster of Paris. In fact, the plaster absorbs the water from the mixture and causes it to set. (4) Colors, such as carmine (acid-free red water color in tubes), may be incorporated in the vulcanized latex to give it a flesh tone before it is poured. There is enough yellow in the set Vultex to give it a fleshlike tone when the carmine is added. Zinc oxide may be added to the liquid vulcanized latex to prevent the resulting prosthesis from becoming a too dark yellow. (5) Shrinkage does not occur as much in highly concentrated vulcanized latex mixtures as in thin mixtures. Unless the mold is tamped or jarred during the pouring, the resulting prosthesis may be partially hollow. Fillers can be added to make the mixture thicker.

*This material may be obtained from R. W. Edmonds, Manufacturers' Agent, 401 Water Street, Baltimore, Md.

These increase the tendency toward a hollow casting. Of course, large prostheses, such as hands, should be hollow.

Should light metals, such as aluminum and silver or their alloys, be chosen for the prosthesis, the metal may be shaped to the correct form in a swedge mold or die and counter die. These may be of plaster. The formation of the metal must be taken in easy stages, with numerous counterdies of different levels to prevent tearing of the metal. The metal is then annealed between each stage.



A

B

FIG. 5—A. If the red wax nose fits perfectly the same mold used for casting the red wax may be used for casting the final positive material. In this illustration the final cast has been attached to the spectacles. An artificial mustache has been attached to the final cast.

B A front view of the same patient.

Metal prosthesis is considered inferior to other substances for attachment to the human body. However, should the worker care to pursue this subject he may find valuable information in the dental literature.

I have discussed moulage prosthesis more fully in the book, *Molding and Casting*. However, since the publication of this book the Mayo Clinic has published two articles on the subject^{6,7} which give specific formulas for compounding rubber prostheses from vulcanized latex. I find that a single formula does not fit every case. If the worker understands his materials, he is likely to vary them to fit individual cases.

The final prosthesis is fixed to the patient with the gum mastic solution previously mentioned or with some mechanical device as eyeglasses or eyeglass frames. The prosthesis in Fig. 5 was riveted to the nosepiece of the eyeglass frame.

Sometimes it is desirable to touch up a prosthetic appliance with additional color because its monochrome effect may not be entirely realistic. If the original

is in celluloid, a small amount of transparent oil color (phototinting color) may be mixed with acetone to be used in the coloring. This acetone softens the celluloid sufficiently to allow some of the dye to penetrate the surface without destroying the skin effect. If a synthetic plastic is used, its solvent can be incorporated with a transparent oil color in the same manner to give additional tints. If the prosthesis is in rubber, benzene or carbon tetrachloride may be incorporated with the transparent oil color. These solvents cause the prosthesis to be slightly sticky immediately after they are used, but they dry in a short while. The worker should realize that to do this work successfully a certain amount of practice and skill is necessary. He should test his tinting ability on objects that have no value. It is possible that his first attempts at prosthetic work will be failures. The objects should be saved to be used in testing other processes in the work, such as tinting.

After the prosthesis is put in place on the patient, cosmetics can be used successfully in making the prosthesis blend with the surrounding tissue. The preparation called "Covermark"* is excellent for this purpose. Any small cracks between the prosthesis and the surrounding tissue may be filled with a putty made by mixing vanishing cream and talcum powder. However, if such cracks are quite obvious it is logical that the prosthesis should be made over. A well-made prosthesis should not be detected at a distance of three feet under ordinary lighting conditions, such as daylight in the average room or artificial light at night.

REFERENCES

1. Kazanjian, V. H., Rowe, A. T., and Young, H. A.: Prosthesis of the Mouth and Face, *J. Dent. Research* 12: 651, 1932.
2. Clarke, C. D.: *Molding and Casting, Its Technique and Application*, Baltimore, 1935, John D. Lucas Co.
3. Lederer, F. L.: Prosthetic Aids in Reconstructive Surgery About the Head, *Arch. Otolaryng.* 8: 531, 1928.
4. Van Dijk, J. A.: *Aeta oto-laryng.*, Part 1.
5. Bercowitseh, G. G.: Facial Restoration, *Dental Cosmos* 70: 167, 1928.
6. Bulbulian, A. H.: An Improved Technic for Prosthetic Restoration of Facial Defects by Use of a Latex Compound, *Proc. Staff Meet., Mayo Clin.* 14: 433, 1939.
7. Bulbulian, A. H.: Prosthetic Restorations of Facial Defects by Use of Latex Compound: A Further Detailed Description of the Technic Used, *Proc. Staff Meet., Mayo Clin.* 14: 721, 1939.

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DEPARTMENT OF ABSTRACTS AND REVIEWS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

PURPURA, The Present Status of Thrombocytopenic, Wiseman, B. K., Doan, C. A., and Wilson, S. J. J. A. M. A. 115: 8, 1940.

No single theory for the production of essential thrombocytopenic purpura adequately accounts for all the known facts relating to this disease; however, that of splenic thrombolytic appears to violate the fewest.

In differential diagnosis aspiration of bone marrow to rule out leucopenic leucemia, aplastic anemia, pernicious anemia, and neoplasia is at times essential. Historical facts relating to drugs and present or recent contact with certain infectious diseases are most important.

In essential thrombocytopenia the only measure known that will restore the platelet level is splenectomy. The more pronounced the bleeding tendency the more urgent the indication for surgery.

No case of symptomatic thrombocytopenia, regardless of the degree of bleeding, should be submitted to surgery.

LEAD, Absorption and Intoxication in Man Unassociated With Occupational or Industrial Hazards, Hansmann, G. H., and Perry, M. C. Arch. Path. 30: 226, 1940.

The analysis of the data obtained may be divided into facts derived from the data and suggestions for consideration contained in the data.

A. Facts derived from the data:

Examinations of tissues for lead absorption were made on 48 bodies, the ages of which ranged from 11 weeks' gestation to 93 years of age.

The amount of lead in the ribs varied from 23.058 mg. to 0.00 mg. per hundred grams.

The amount of lead in the liver varied from 21.033 mg. to 0.00 mg. per hundred grams.

The lead content is expressed in milligrams per hundred grams of dried tissue.

There appeared to be no relationship between the amount of lead absorbed and the age, except that those subjects containing no lead were either fetuses or children under 12 years of age.

The amount of lead in the liver may exceed that in the rib during prolonged metabolic disturbances, severe infections, and less acute progressive fatal illness.

A mother who has absorbed lead will excrete increasing amounts of lead during gestation, the excretion of which parallels the skeletal growth of the fetus.

The analysis of entire fetuses, the ages ranging from 11 to 24 weeks' gestation, revealed lead in 62.5 per cent of them. The amount in 25 per cent of these bodies may be considered hazardous.

Eighty per cent of fetuses from 4½ months to term had lead in the rib or the liver or in both organs.

All subjects over 12 years of age or 90 per cent of those between birth and 93 years of age revealed evidence of lead absorption.

A review of the results recorded in the literature with which our results were integrated, has established by spectrographic, colorimetric, and titrametric methods that lead absorption is a normal consideration.

The cases were all drawn from Milwaukee and its vicinity.

B. Suggestions for consideration contained in the data:

Lead absorption may act as an unrecognized factor in various diseases in persons who cannot trace their exposure to lead.

Aged persons, who frequently lose, often quite rapidly, upward of 50 per cent of their skeletal calcium, may suffer symptoms of intoxication from the lead which is concurrently released, provided the skeleton is heavily leaded.

Women may suffer from lead intoxication during gestation, owing to the fact that much lead is released from a heavily leaded skeleton during gestation. This may be responsible for some of the anemias closely resembling pernicious anemia which occur during and shortly after pregnancy.

The fetus is likewise exposed to lead hazards, which may result in intoxication of the fetus or expulsion of the fetus as a result of the action of lead on the uterus.

The patient with a heavily leaded skeleton may suffer from symptoms of lead intoxication during uncontrolled metabolic diseases, severe infections, or prolonged, progressive illness, owing to the fact that lead is mobilized from the skeleton and fixed by the organs.

Lead may be an important cause of abortion during the first three months of gestation.

The development of the fetal skeleton may protect the fetus by withdrawal of lead from the circulation.

Lead absorption may become an individual problem and the concern of every physician.

JAUNDICE, Renal Lesions Associated With Deep, Ayer, D. Arch. Path. 30: 26, 1940.

In infants with long-sustained uncomplicated jaundice, due to congenital atresia of the bile ducts, renal lesions occur without producing oliguria. These lesions consist of focal exudative changes, obstruction of tubules by casts, and phagocytosis of cast material by epithelial cells. These lesions are duplicated in the kidneys from patients with transfusion reaction and the hepatorenal syndrome.

SULFANILAMIDE, A Micro Bedside Test for Determination of, in Body Fluids, Schoeffel, E. W. J. A. M. A. 115: 122, 1940.

A. Place a drop of the body fluid under examination (approximately from 0.01 to 0.05 c.c.) with calibrated pipettes of the blood pipette type in the depression of a hanging-drop slide. (The liquid may be dried by air current. If the material must be shipped, the dried substance is covered with a drop (from 0.050 to 0.075 c.c.) of liquid petrolatum U.S.P. and the cover slip fastened on with a seal.) Place all slides on a clean white towel.

B. Add 0.02 c.c. of 4 normal hydrochloric acid and 0.02 c.c. of 10 per cent trichloroacetic acid to the material in the depression. Break up and stir thoroughly with a glass thread. For total sulfanilamide heat over an alcohol lamp to near dryness and repeat step B.

C. Add 0.03 c.c. of 0.1 per cent sodium nitrite solution and stir the mixture thoroughly for thirty seconds with a glass rod (with amounts of free sulfanilamide in excess of 20 mg. for 100 c.c. use 0.05 c.c.).

D. Add 0.02 c.c. of a solution containing 0.1 per cent ammonium sulfamate and stir the solution thoroughly (for larger amounts of sodium nitrite use corresponding amounts of the sulfamate solution).

E. Add 0.02 c.c. of 0.4 per cent aqueous solution of N(1-naphthyl)-ethylenediamine dihydrochloride (Marshall's reagent; the solution should be kept in a dark brown bottle and only enough made up to last two days). Stir the mixture thoroughly.

F. Prepare standards (as described under standard reagents and apparatus to be used for the test) containing 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12.5, and 15 mg. per hundred cubic centimeters. If testing for sulfanilamide, use sulfanilamide in the standards; if testing for sulfapyridine or sulfathiazole, use sulfapyridine or sulfathiazole standards, respectively. Such standards are good for one week if kept in an icebox and away from direct light. The standards may be conveniently prepared by carefully diluting a larger amount.

G. Insert in the depression containing the now red solution a strip of filter paper and let the liquid spread by capillary action over one end of the paper (precipitated protein material does not interfere with the test). Compare the unknown samples with the standards. The developed color depends upon the drug used. It is necessary for the technician to know which of the sulfanilamido derivatives were used. It is important to know whether other therapeutic agents were also used. Especially with specimens of urine, a slight color varia-

tion may be noticed, depending upon various diazotizable substances. Since no fractions are to be determined and only round figures are given (2.5, 4, and 7.5 mg. per hundred cubic centimeters), no strong interference exists. The color on the filter paper will slowly fade but will hold distinctive and discernible hues for a period of days when compared with standards prepared on the same day and kept under the same conditions. Actual color charts may also be prepared to facilitate the determination at the bedside. In general for ease of reproduction the final amount of liquid in the depression should not exceed the approximate amounts of from 0.125 to 0.150 c.c.

HEPATIC DISEASE, Clinical Value of Determination of Cholesterol Esters of Blood in,
Greene, C. H., Hotz, R., and Leahy, E. Arch. Int. Med. 65: 1130, 1940.

The value for cholesterol in the blood of adults varies between 150 and 230 mg. per hundred cubic centimeters, whereas the combined cholesterol (esters) varies between 60 and 120 mg. The ratio of the combined to the total blood cholesterol is quite constant—between 40 and 52 per cent.

In this series, patients with evident hepatic damage had a decreased amount of combined cholesterol in the blood. This decrease was sometimes, but not always, associated with a decrease in the total cholesterol. The ratio per se was not of as great diagnostic usefulness as was the total amount of esters present in the blood.

In uncomplicated obstructive jaundice the combined cholesterol tends to rise in proportion to the rise in total cholesterol; but in hepatic disease the cholesterol esters tend to disappear from the blood regardless of the behavior of the total cholesterol.

In a given case a progressive decrease in the values for the combined cholesterol of the blood signifies a poor prognosis, whereas a progressive increase signifies a good prognosis.

The determination of the combined cholesterol of the blood is of great value in determining the prognosis of surgical treatment of patients with disease of the biliary tract.

TISSUE: Hamdi's Preserving Solution, Cambel, P. Arch. Path. 29: 813, 1940.

The specimen to be preserved is fixed in a 10 to 20 per cent dilution of the 40 per cent stock solution on the market. The larger and more solid the specimen (brain, liver, large spleen, large tumor) the higher the percentage of stock solution that may be used. After the specimen has been thoroughly fixed and prepared, it is left in running tap water for at least twenty-four hours; then it is placed in a highly hypertonic (about 50 per cent) salt solution prepared with clean tap water. Here it is left for two to five days. The hypertonic salt solution penetrates the organ, drives out the formaldehyde solution, causes a slight swelling, which removes the shrinkage due to the formaldehyde solution and increases the weight of the specimen, so that the lungs, for instance, do not float when placed in the preserving fluid. It helps, moreover, to prevent the growth of molds. After the organ has been rinsed in running water, it is placed directly into Hamdi's solution, the formula of which is as follows:

Sodium sulfate (pure)	5 Gm.
Salt (pure)	100 Gm.
Clear tap water containing no organic impurities	1,000 Gm.
Glycerin	50 Gm.

The solution is clear, practically colorless. It may be yellowish if the glycerin is yellowish. Icteric organs sometimes give it an icteric tint. After a couple of years it may become slightly yellowish, especially when the specimen is exposed to much light.

A few drops of a saturated camphor solution in 96 per cent alcohol is added. The white precipitate that forms is dissipated by superficial stirring with a glass rod. Then the glass cover is sealed on the container.

If the seal loosens and molds form, and even if the color of the specimen is spoiled on account of the molds, it is sufficient to leave the specimen for half an hour to a few hours in running water and in a jar into which a few crystals of potassium permanganate have been placed. The organ is then rinsed in running water, treated with salt water or not, replaced in fresh Hamdi solution, and the jar resealed.

The advantages of Hamdi's solution are: It does not necessitate a preliminary treatment of specimens with alcohol as with most of the preserving solutions now in use. It preserves the specimens practically in their natural condition; it does not destroy the red blood corpuscles.

HYPERTENSION: I. Effect of Nephrectomy Upon, Associated With Organic Renal Disease, Schroeder, H. A., and Fish, G. W. Am. J. M. Sc. 199: 601, 1940.

Seven patients exhibiting arterial hypertension associated with organic renal disease have been subjected to nephrectomy. Two were markedly improved, and 2 slightly improved, but all remain actually or potentially hypertensive.

This form of therapy may prove of benefit, but, it seems, only in patients in whom the existence of hypertension is of short duration and in whom arteriolar sclerosis of the other kidney is not advanced. Its use is limited, therefore, to a small number of individuals.

As criteria for selection of cases for this form of therapy the authors suggest:

1. The onset of hypertension should be known to have occurred recently (arbitrarily two years).
2. The renal lesion should be confined to one kidney and should be of such a nature that diminution of function has occurred in that kidney.
3. Renal functions, as measured by the ability of both kidneys to concentrate urine and by the test of the clearance of urea, should be within normal limits.
4. Retinitis should be absent, and changes in the caliber of the vessels of the retina should be minimal.
5. Arterial pressure should be persistently elevated.

II. ARTERIAL HYPERTENSION, The Role of the Kidney in the Pathogenesis of, Dicker, E. Am. J. M. Sc. 199: 616, 1940.

For the kidneys to be able to cause hypertension their circulation must be restricted; all the other renal and urinary manifestations are secondary, independent, and incapable of playing a part in the production and maintenance of the hypertension.

III. ESSENTIAL HYPERTENSION: Comparison of the Hypertensive and Non-Hypertensive Phases Following Coronary Thrombosis, Gross, H., and Engelberg, H. Am. J. M. Sc. 199: 621, 1940.

An analysis is presented of 100 autopsied cases of hypertension and severe coronary artery disease studied for the effect of the blood pressure on the subsequent course. All the patients had cardiac hypertrophy and marked myocardial damage.

Ninety patients had chronic congestive heart failure. The high incidence of heart failure is partly due to the type of patient admitted to Montefiore Hospital. The onset of heart failure frequently followed an acute coronary occlusion. This occurrence was so striking that in cases of chronic coronary sclerosis, when heart failure begins rather abruptly, a silent coronary occlusion should be suspected.

There were 24 persons with terminal acute coronary closure and in these the course of blood pressure was known for at least one year prior to death. Fifteen had hypertension persisting up to the final closure, 7 had low blood pressure for several months prior to the closure, and in 2 the blood pressure varied in the preceding year.

Analysis of the course of blood pressure subsequent to acute coronary occlusion was made. Eighteen persons had persistent hypertension (after recovery from the initial drop), 12 had permanently low blood pressure, and 10 had variable pressure. The same variations in the course of hypertension occurred when no acute occlusion could be diagnosed clinically with certainty.

Twenty-one of the 100 patients died suddenly. Many of these had the clinical picture of acute coronary thrombosis, but in only four of this group was a terminal closure found following coronary thrombosis.

The subsequent blood pressure in hypertensive patients following coronary thrombosis had no effect on longevity, or on the occurrence, severity, and duration of heart failure. Neither was there a definite relationship between the course of blood pressure and the heart weight and the duration of failure. Physiologic factors undoubtedly play a role adjusting the work of the heart to a restoration or a permanent fall in the blood pressure. These factors have been discussed.

SULFANILAMIDE, Relation of p-Aminobenzoic Acid to the Mechanism of the Action of, Woods, D. D. Brit. J. Exper. Path. 21: 74, 1940.

Yeast extracts contain a substance which reverses the inhibitory action of sulfanilamide on the growth of hemolytic streptococci.

Examination of the chemical properties of this substance and its behavior in growth tests suggested that it might be chemically related to sulfanilamide.

p-Aminobenzoic acid has high activity in antagonizing sulfanilamide inhibition.

There is strong circumstantial evidence that the yeast factor may be p-aminobenzoic acid.

SULFANILAMIDE, The Inhibition of the Action of, in Mice by p-Aminobenzoic Acid, Selbie, F. R. Brit. J. Exper. Path. 21: 90, 1940.

The experiments described show that the therapeutic action of sulfanilamide in mice infected with streptococci can be inhibited by p-aminobenzoic acid in the same way as its antistreptococcal action is inhibited in bacterial cultures. The mechanism of its action in vivo would therefore appear to be similar, if not identical, to its action in vitro. Woods (1940) has suggested that p-aminobenzoic acid or some similar substance is essential for the growth of bacteria and that its utilization can be blocked by sulfanilamide. Many of the discrepancies which have arisen in the course of the work on the sulfanilamide drugs can be explained as due to variations in the amount of the essential substance available in the bacterial cells or in their environment (blood stream, tissues, etc.). It would also appear probable that the lag phase in the action of sulfanilamide coincides with the slow exhaustion of the essential substance.

TRICHINELLA SPIRALIS, Early Mild Infestation With, Andes, J. E., Greene, R. A., and Breazeale, E. L. J. A. M. A. 144: 2272, 1940.

Of ten patients believed to have been infested with *Trichinella spiralis*, all but one gave a history of eating pork from one to three days before the onset.

The condition of eight of these patients was diagnosed within the first three days of the onset. The most common symptoms were malaise, abdominal discomfort, fever, headache, and edema around the eyes. All showed a definite eosinophilia and all tested gave a positive skin reaction and trichinella antigen. Administration of tetrachlorethylene was followed by complete and immediate recovery.

The two patients consulting the authors six and seven days after the onset contracted muscle pain and tenderness; the third patient showed a fairly long drawn out convalescence.

The eosinophile count of the blood was shown to rise continually during the day, being highest in the evening.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor,
Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

The Author Publisher Printer Complex*

A WELL-WRITTEN booklet on the preparation of manuscript, correction of proof, and related aspects of writing and publishing a book, the title might more accurately have been "Advice From a Publisher to His Authors."

The author who has read this book will have a much clearer understanding of the problems which his publisher must solve and the decisions which he must make, many of which might otherwise have appeared unjustified.

This small work should be highly recommended to all who contemplate writing a book or an essay. It will be enjoyed equally by those who have written in the past.

Directory of Medical Specialists†

ENTERPRISING firms have in the past attempted to prepare reference catalogues of physicians in the various specialties in the United States. Some have catalogued any and all who were willing to pay for a copy of the finished book, while others have attempted some manner of selection, such as recommendation by recognized leading physicians in different localities.

None of these has been satisfactory. Those who have had occasion to investigate the qualifications of doctors in distant localities have had to rely chiefly on the sparse information available in the Directory of the American Medical Association.

With the advent of certification in the specialties after adequate examination by official boards, a satisfactory standard for inclusion in a directory of this sort has for the first time become available. The Directory of Medical Specialists includes all persons in the United States and its possessions and in Canada who are diplomats of the official boards in the various specialties. The Directory is, therefore, authentic and reliable. The Board of Editors comprises the secretaries of the several specialty boards.

Approximately 14,400 diplomas certified by the twelve special American boards and one of the two affiliate boards are listed. Cross indexing, geographical and alphabetical, is adequate.

Twice on the day of arrival of the book the reviewer had occasion to use it, thus promptly discovering that it adequately fulfills its purpose.

*The Author Publisher Printer Complex. By Robert S. Gill. Cloth, 76 pages, \$1.00. Williams & Wilkins Co., Baltimore, Md., 1940.

†Directory of Medical Specialists. Certified by American Boards. By Paul Titus, M.D., Directing Editor. Cloth, 1,573 pages, \$5.00. Published for the Advisory Board for Medical Specialties, Columbia University Press, New York, N. Y., 1940.

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PROGRESS

CLINICAL ASPECTS OF SULFAPYRIDINE THERAPY*

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PNEUMONIA has long been recognized as one of the more important of the lethal afflictions of mankind.

From Hippocrates,¹ who wrote of the importance of the "cough" of the sputum; and Aretaeus,² who began his discussion with these words: "Animals live by two principal things, food and breath (pneuma); of these by far the most important is the respiration, for if it be stopped, the man will not endure long but immediately dies"; on to Osler's classic description of this disease as "Captain of the Men of Death," pneumonia has attracted the attention of the physician and investigator throughout the ages.

But despite the work of innumerable investigators, pneumonia for centuries remained a disease before which the physician stood almost without resource; for progress in medicine, though steady and persistent, often seems intolerably slow.

Not until the advent of type-specific immune sera was there any marked advance in the therapy of pneumonia, and great though this discovery was, the victory was but partial. For, though it was learned that the race of pneumococci embraced 32 serologically separable types—perhaps more—for but relatively few of these did it seem possible to develop therapeutically effective antisera.

Attention was focused, therefore, upon developing a specific and effective chemotherapy; not necessarily what Ehrlich defined as a "*therapia sterilisans magna*" but some drug which per se, or through its derivatives, might offer definite and demonstrable hope for the consistently efficient management and control of pneumonia in all its varied bacteriologic entities. Thus, for many years, this drug or that, this compound or the other, flourished for a while and, under the acid test of time and trial, passed on to obscurity.

*From the Laboratories, Atlantic City Hospital.

Appreciation of great discoveries in medicine is more often gradual than dramatically sudden; indeed, they often pass relatively unnoticed until fortuitous circumstances bring them to light.

Thus it was with the "Quellung" reaction, described by Neufeld, which, though now an essential prerequisite to the intelligent treatment of pneumonia, lay dormant for a quarter of a century. Thus it was also with the discovery of sulfanilamide which, though elaborated in 1908, remained with all its remarkable qualities unheralded and unsung until comparatively recent times.

As its widening range of capabilities became more and more apparent, it was inevitable that attempts should be made to find in sulfanilamide, or in its derivatives, some drug which, effective against the pneumococcus, would also possess a chemotherapeutic index rendering it safe for clinical use.

It is needless to list the innumerable substances synthesized in these attempts. If the resultant compound proved effective against the pneumococcus in experimental infections, all too often the chemotherapeutic index—the margin between the toxic and the therapeutically efficient dose—was too small for safety.

It was not until the report of Whitby³ in 1938—it seems difficult to appreciate that it was only two and a half years ago—of the apparent efficiency of 2-sulfanilylaminopyridine, a substance elaborated by Ewins and Phillips in England, that there seemed some promise that the long-sought goal had been reached.

Whitby first reported the effectiveness of this compound against the pneumococcus, but it was the report of Evans and Gaisford⁴ in the same year which first showed its application to the treatment of lobar pneumonia. Since then innumerable reports concerned with it have been written, with the end not yet in sight.

Though efficient chemotherapy for pneumonia seems in large measure to have been at last achieved, oddly enough we are still largely at a loss for a satisfactory explanation for, or a definite understanding of, the mode of action of sulfapyridine upon the pneumococcus. That it has an action the clinical response following its administration in pneumonia proves beyond dispute. That this clinical effect is dependent upon some mechanism yet to be explained is equally clearly shown by various laboratory and experimental studies.

It is well recognized that bacterial infection is primarily dependent upon the concomitant influence of several factors, some of primary and essential, others of ancillary, importance.

In addition to the inherent and acquired resistance of the patient and his tissues, the virulence, aggressiveness, and toxicity of the invading bacteria must all be taken into account. Only when a suitable balance or imbalance between these varied factors is present does infection occur.

When chemotherapy is effective, it is logical to look for the reason in its effect upon bacteria, and it is ordinarily to be expected that that effect will be mainly, if not entirely, bactericidal.

But the action of sulfanilamide and its derivatives, sulfapyridine among them, upon the pneumococcus is not bactericidal but apparently merely bac-

teristatic. For while the pneumococcus remains alive and propagates itself in the presence of sulfapyridine, there is a definite lag in its growth, which appears to be definitely inhibited, establishing the drug as bacteriostatic rather than bactericidal.

We do not know how this bacteriostatic effect comes about; for while there are many theories and hypotheses, all must be regarded as speculative and unproved. This is, perhaps, not altogether surprising in view of the fact that the pneumococcus itself still remains in many respects somewhat of a bacteriologic puzzle.

Perhaps the most important advance toward an understanding of the pathogenicity of the pneumococcus was the discovery that its most important constituent, and the one most largely concerned in its action upon the tissues, resides in its capsule, the "specific soluble carbohydrate substance," as it is now called. Unfortunately, this substance is soluble and passes into the blood stream. In many respects the severity of the pneumonic infection is rather directly proportionate to the concentration in, and distribution of, this substance by the circulation.

We are still uncertain of the relationship between this capsular polysaccharide and the invasiveness of a particular strain of pneumococci. We do not know whether the polysaccharide may properly be regarded as the pneumococcus toxin, or whether the pathogenic ability and toxicity of the pneumococcus resides solely in its capsular substance or in part—or at all—in its somatic protein substance. These are but a few of the problems to be solved.

Granting the bacteriostatic effect of sulfapyridine upon the pneumococcus, what, if any, is the relationship and the importance of this effect to the clinical results obtained?

Is the bacteriostatic effect the ultimate explanation? Or does the bacteriostasis simply allow time for mobilization of the defensive mechanism of the patient so that the neutralization of toxic substances and the elimination of the pneumococcus by phagocytosis, bacteriolysis, and the contributory effect of opsonins may be effectively exerted?

While any or all of these may take place, or may be in some measure determined by bacteriostasis and thus play some part in recovery, it is difficult to believe that this is the whole story.

In view of the importance of the specific capsular polysaccharide in determining the pathogenicity and toxicity of the pneumococcus, does any evidence suggest a relationship between the clinical effects of sulfapyridine and some possible effect upon the capsular substance, its elaboration, or its concentration in the blood stream? May sulfapyridine prevent or inhibit the formation of capsular substance or in any way immobilize it or render it innocuous or relatively so? Any such assumption must postulate an effect upon the pneumococcus capsule consequent upon exposure to sulfapyridine. Is there any evidence suggesting such a possibility? Here at last we find a question to which a conditioned and reserved answer may be made, based upon observations by various workers in connection with pneumococcus typing by the Neufeld method.

As everyone now knows, type specificity is demonstrable in the Neufeld method by the occurrence of the Quellung reaction evidenced by a marked swelling of the pneumococcus capsule when brought in contact with its homologous immune serum.

Failure to type the pneumococcus may be dependent upon technical errors when the method is applied by those unskilled in bacteriologic procedures; it may be influenced by dissociation of the pneumococcus; certain types if not thereby shown to be identical, certainly show cross reactions with typing sera; failure to secure type differentiation may suggest there are more than 32 types; there is also definite and cumulative evidence that pneumococcus typing in the presence of sulfapyridine is not infrequently difficult and unsatisfactory, if not impossible.

It must be admitted, however, that the relationship of sulfapyridine to anomalous results in pneumococcus typing is still *sub judice*. Bullova, Osgood, Bukantz, and Brownlee,⁵ from a study of pneumococcus-infected marrow cultures, report that while pneumococci exposed to sulfapyridine may become distorted, develop long chains, and stain irregularly, they suffer no loss of capsule or type specificity.

Now, as the Quellung reaction upon which pneumococcus typing depends arises from an effect upon the capsule, if this effect is interfered with in the presence of sulfapyridine, it seems logical to postulate some effect of the drug upon the capsule. If this be so, then various speculations arise as to some possible relation to the specific capsular substance.

But again we encounter a maze of speculative hypotheses. Does sulfapyridine affect the permeability of the capsule, and, if so, would this increase the vulnerability of the pneumococcus to antibodies such as opsonins and hence to phagocytosis? Is the effect something other than some change in permeability? Does some change occur in the reactive ability of the capsule, and, if so, what is its nature? Or is there some alteration in the elaboration, mobilization, or solubility of the specific capsular substance? To none of these queries can a satisfactory or definitive answer yet be given.

It is both logical and empirical to assume a definite relationship between the chemotherapeutic efficiency and the concentration of the drug in question. As this has been shown to be in a large measure true of sulfanilamide, it was logical to assume that it must also hold true for sulfapyridine, the effective concentration in the blood being generally regarded as between 4 and 10 mg. per 100 c.c., with the latter as the upper limit of safety. However, there are fairly definite indications that suggest a lack of definite correlation between the blood concentration of sulfapyridine and the clinical result consequent upon its use in pneumonia.

Illustrative of such reports is that by St. George, Kraetzer, and Magee⁶ of a carefully controlled series of 50 unselected cases. Their tables show concentrations of sulfanilamide in the blood varying from 1.3 to 6.5 mg. per 100 c.c.; the latter being the highest they obtained. But, despite this variation within fairly wide limits, there was no apparent correlation with the ultimate clinical result nor was the incidence of recovery in any way definitely proportionate to the blood concentration.

It is recognized that the blood concentration is affected by factors such as nausea and vomiting, influencing the rapidity and degree of absorption, and also perhaps by others not yet clearly understood that may affect the degree to which the drug is acetylated or conjugated. Nevertheless, this and other similar reports are significant, even though at first they seem merely to further beloud an already obscure picture.

The intelligent consideration of any therapeutic agent must embrace not only its therapeutic effects, but also its nonspecific and undesirable by-effects; in other words, its toxicity for the human being.

Here we enter upon somewhat less uncertain ground for, though we may not always possess a clear explanation for their occurrence, we have cumulative and increasing clinical evidence of definitely toxic and undesirable by-effects of sulfapyridine for which it is necessary to be on guard and which, in any degree, it is highly desirable to avoid.

Remembering that sulfapyridine is synthesized from aminopyridine, it is not altogether amazing that there is some reason to believe that certain individuals, fortunately seldom encountered, may possess an inherent sensitivity to the drug which renders them more vulnerable to undesirable toxic effects. Because sulfapyridine is regarded as of relatively low toxicity, it does not follow that it can be safely administered without regard to the various toxic manifestations which may appear, sometimes insidiously but not infrequently with disturbing rapidity.

Cyanosis is a very common occurrence after the administration of sulfapyridine but, even though marked, is rather generally regarded as of minor significance and not per se indicating withdrawal of the drug. As might be expected, there is some uncertainty whether sulfapyridine cyanosis depends upon sulfhemoglobinemia or methemoglobinemia. Cyanosis is not infrequently a symptom in pneumonia and may be present before, and hence independent of, the administration of sulfapyridine. But since the cyanosis in pneumonia is directly related to the oxygen saturation of the arterial blood, any increase consequent upon the administration of sulfapyridine cannot always be regarded with entire equanimity and unconcern.

The recent observation by Doughty,⁷ corroborating the report by McGinty, Lewis and Holtzelaw,⁸ that the cyanosis and the usual concomitant headache, weakness, and nausea following the administration of sulfanilamide may be effectually relieved by the administration of 20 mg. of nicotinic acid three times daily, is of definite value and may furnish a clue to the mechanism of these complications.

As the oral administration of sulfapyridine is at times impractical or impossible, the recent report of Haviland and Blake⁹ is of interest. These investigators have found that a 0.15 per cent solution of sulfapyridine in normal (0.85 per cent) saline, or a 0.2 per cent solution in 5 per cent glucose solution, or in equal parts of normal saline and 5 per cent glucose, may be given intravenously without undesirable effects as long as the solutions are not allowed to cool below room temperature. The solutions are made by boiling and are allowed to cool spontaneously to room temperature. Such solutions have been given intravenously, subcutaneously, and within the arachnoid space and pleural cavity.

The main objection the authors cite is that large amounts (up to one or two liters) must be administered to procure adequate concentration. As in the presence of loss of fluid, salt, and glucose because of inadequate intake in comatose patients, or through vomiting, these substances may thus be replaced, the objection is of minor importance.

That sulfapyridine per se may produce a febrile reaction has been shown by many observers. It is important to differentiate this so-called "drug fever" from febrile exacerbations consequent upon the development of various complications. This may not always be easy for, though Graham, Warner, Dauphinee and Dickson¹⁰ report that drug fever is not accompanied by leucocytosis, this has not been the consistent experience of others.

In keeping with the observation that vomiting following the administration of sulfapyridine depends largely, if not entirely, upon central nervous system irritation, it has been recorded that various other central nervous system disturbances may occur. The more common of these are vertigo, headache, malaise, and mental depression. Occasionally, excitement of a degree severe enough to be classed as a psychosis occurs in approximately 4 per cent of cases. Fortunately these reactions are transient and disappear promptly on withdrawal of the drug.

Reference has already been made to the wide variation in the blood concentration and to the difficulty of maintaining the blood concentration at any set level with any degree of constancy. Many factors are probably concerned: variations in absorption, variations in the excretion rate, and variations in the degree and rapidity with which changes in the composition and structure of the drug occur after absorption, notably in the liver.

Just as bacteria entering the blood stream do not remain there as particulate entities continually traversing the circulatory paths but are rather rapidly removed through the interlocking activities of various mechanisms, so chemotherapeutic agents entering the blood stream are likewise subject to varying influences. Obviously, they are at once diluted by the blood volume; they may in part combine with its protein or other constituents, with their activities thereby decreased, if not entirely inhibited; and they are frequently altered by the detoxifying function of the liver.

It is known that compounds having a free para-amino group—of which sulfapyridine is one—undergo acetylation in the liver as a result of which the free amino group is blocked. Sulfapyridine may thus be rendered more or less inert after absorption. Fortunately, though this reaction occurs in the liver, evidence of tissue irritation as a by-product is exceedingly rare, so that toxic hepatitis and jaundice are very uncommon.

Like many other drugs sulfapyridine may produce a drug rash, the particular importance of which is that, as such patients appear to be definitely photosensitive, the severity of rash may be greatly intensified by exposure to light, particularly strong sunlight.

Since sulfapyridine in both its free and conjugated (acetylated) form is excreted almost entirely by the kidney, and since it may be present in the urine in concentrations much greater than its solubility can account for,^{11, 12} the occurrence of renal complications would not be unexpected. Thus a temporary de-

crease in the urica clearance, regarded as a toxic manifestation, has been reported by MacLeod,¹³ and hematuria, generally microscopic, though occasionally macroscopic, has been noted by many observers.

The incidence of hematuria is low—0.8 per cent in 2,300 cases, in one report. Since it shows evidence of renal irritation and damage, it indicates the necessity for frequent and periodic urinalyses during the course of sulfapyridine therapy. It is also well to maintain a high fluid intake to dilute the concentration of the drug in the kidney and lead to its rapid elimination. As sulfapyridine is more soluble in alkaline than in an acid solution, the proposal of Long and Wood¹⁴ that the urine be kept alkaline with sodium bicarbonate is worthy of note, even though it is purely empirical and as yet of unproved efficacy.

Of greater interest and more importance are the reports—relatively few but increasing in number—indicating the possibility of calculus formation as an aftermath of sulfapyridine therapy. Calculus formation in animals—the calculi being composed chiefly of acetylsulfapyridine—has been demonstrated experimentally by Toomey,¹⁵ Antopol and Robinson,¹⁶ and by Gross and associates.¹⁷⁻²⁰

It is not yet possible to determine accurately how frequently this complication occurs, but some evidence suggests that the incidence may be greater in children than in adults. That, rarely, grave and even fatal consequences may follow is shown by a fatality reported by Long and Wood,¹⁴ and one by Tsao, McCracken, Chen, Kuo, and Dale²² following renal blockage and uremia; in view of these fatalities the importance of routine urinalyses with attention to the presence of red blood cells and sulfapyridine crystals in the urinary sediment is obvious.

By far the most important and most dangerous complications of sulfapyridine therapy are those resulting from depression of the hematopoietic system and manifested by the occurrence of hemolytic anemia and agranulocytic neutropenia.

While both of these are important, the anemia—while not infrequent and tending to be progressive—is comparatively the less dangerous, since it is practically always controllable by transfusion and withdrawal of the drug. Although apparently essentially hemolytic in type, the anemia may vary in its manifestations, in some cases resembling a definite chloro-anemia; in other instances the reduction in the red blood cell count is disproportionate to the reduction in hemoglobin. Present opinion inclines to the belief that, where the need for continued administration is urgent, anemia of itself is not a mandatory indication for withdrawal of the drug unless the anemia reaches a degree dangerous per se, and particularly when it cannot be controlled by transfusion.

Agranulocytic neutropenia may take place early after the administration of only a small amount of sulfapyridine. Usually, once begun, it progresses rapidly. Its importance lies in the fact that marked leucopenia invites invasion of the tissues by opportunist bacteria, normally quiescent and particularly present in large numbers and varieties on mucous surfaces (mouth, rectum, vagina), leading to necrosing and extensive tissue damage and destruction.

It is well recognized that granulocytopenia in malignant degree is the aftermath and expression of profound depression of the bone marrow. But the

majority of reports suggest that this is most likely to occur when the dosage of sulfapyridine has been large and long continued. In common with other forms of grannlocytopenia dependent upon chemical intoxication, as contrasted to the form originally described by Schultz as agranulocytic angina which is now believed to be a filtrable virus disease, that following sulfapyridine therapy is more intense, and more likely to be dangerous in the presence of an individual sensitization or personal idiosyncrasy to the benzol ring. It is interesting to note that there appears to be no evidence that an individual can be sensitized to sulfapyridine itself.²³ Here, again, the importance of routine blood counts is evident, particularly because bone marrow damage may be manifest only after the cessation of sulfapyridine therapy.²⁴⁻²⁶

While this survey has been by no means comprehensive, it may serve to indicate that sulfapyridine therapy is still largely in the stage of clinical experimentation and, therefore, still in a state of flux.

It is unfortunate, in several respects, that the clinical use has far outstripped the careful and controlled laboratory study of sulfanilamide and its derivatives. It has been haphazard and empirical, to some extent based upon "wishful thinking," and has too often failed to appreciate that it is in large measure not clinical therapy, but clinical experimentation. What is the moral?

The moral, as I see it, is that if we are perforce engaged in mass clinical experimentation, we dare not evade the responsibility of so carrying on our investigations as to make them informative and ultimately of value. This means that our use of sulfapyridine and similar compounds that will inevitably follow it should be so safeguarded as to lessen the possibility of undesirable aftermaths, and so managed and controlled as to lead to a better understanding of its action, its capabilities, and the true indications for its use.

We, therefore, list the following desirable, and in some respects essential, adjuncts to the use of sulfapyridine in pneumonia:

1. The sputum should be typed in *every* case *before* the administration of sulfapyridine. Although this drug is not type specific in its action, knowledge of the type of pneumonia may lead to an evaluation of the place of sulfapyridine in treatment; it will give significant information in relation to prognosis; and it will indicate those cases in which the patient may best be served by combined serum and chemotherapy.

2. A blood count should be recorded *before* chemotherapy is begun, and the blood count should be frequently repeated as long as the drug is administered. It may profitably be continued for some days thereafter. It should be done *before* the institution of drug therapy to distinguish between antecedent anemia, leucopenia, or anemia arising from the disease per se and anemia due to drug toxicity.

The blood count should be *repeated* regularly during treatment because both anemia and granulocytopenia may appear insidiously and develop rapidly and progressively. Ravid and Chesner²⁷ have recently reported death from hemolytic anemia and nephrotic uremia on the third day of treatment after the administration of only 8 Gm. of sulfapyridine. As shown by post mortem, the uremia was apparently the aftermath of tubular blockage resulting from hemo-

siderosis and the presence of hemoglobin casts. There was also very probably some degeneration of the renal tubular epithelium reflecting a direct nephrotoxic action of the drug.

The blood should be observed *after* the cessation of chemotherapy because bone marrow damage may appear after the drug has been discontinued.

3. For reasons already discussed in some detail, routine and regularly repeated urinalyses should be a *sine qua non* of sulfapyridine therapy.

4. It is of great value whenever possible to take a blood culture *before* the institution of sulfapyridine therapy. First, because cultures taken *after* administration of the drug may be unreliable; second, because the blood culture, when positive, may confirm the sputum typing; third, because of the prognostic importance of bacteremia as an indication for energetic treatment, the combined use of serum and drug therapy, or perhaps for the use of sodium sulfapyridine intravenously. Bullowa and his associates,²⁸ in a series comprising 324 adults and 113 children, report that their lowest mortality rate in adults was observed in persons treated early (one to four days) with serum and sulfapyridine. The results in children were inconclusive.

5. While there is some uncertainty concerning the clinical importance of determinations of the blood concentration levels during the course of sulfapyridine therapy, whenever possible these should be done routinely at regular intervals to discover their ultimate clinical significance and value.

6. It is well to mix sulfapyridine therapy with a generous measure of the ingredient recommended by Whistler for use on the palette of the artist, namely, brains.

I am aware that in this somewhat discursive presentation, I have developed more problems and envisaged more difficulties than I have been able or, indeed, have attempted to solve. So much so that one might be tempted to echo the words of General Wolfe in one of his dispatches to Lord Pitt: "There is such a choice of difficulties that I find myself at a loss how to determine."

We may also remember the axiom of Oliver Wendell Holmes that, "Knowledge, like timber, should not be used until it is well seasoned."

If, in perspective, the vista seems one of doubt, of difficulty, even of confusion, we need not necessarily be unduly discouraged. Remember that the mills of science, like those of the gods, grind slowly—but they grind! And if, under the keen handling of the wise, the patient and the long enduring, their little grist grows even smaller as the chaff is winnowed away, that which is left is fine and pure and unalloyed, and from it comes strength and stimulus for the fight against disease.

I believe that we are only on the threshold of the successful chemotherapy of pneumonia. Sulfapyridine is but the forerunner of many sulfanilamide compounds to come. We have only to recall that it first became known as M & B 693, indicating that it was only one of many compounds synthesized in the laboratories of May and Baker at Dagenham, England, to realize the patient persistence of the investigator.

We have only begun the conquest of pneumonia, but we can now envision a conquest; heretofore we have had only a nebulous hope. We have taken the

first and the hardest step and may well expect that those to follow will be neither few nor laggard in their coming.

I hope this discussion will not have seemed entirely enveloped in uncertainty and disagreement. If this be so, let me recall the words of Herbert Spencer: "Mankind, in its progress, passes through three stages: the unanimity of the ignorant; the disagreement of the inquiring; and the unanimity of the wise."*

*ADDENDUM: Since this paper was written, two further communications have appeared concerning the mode of action of sulfanilamide.

Lockwood and Lynch²⁰ believe, from their investigations, that the bacteriostatic as well as they state, "very limited bactericidal action in vitro" of sulfanilamide are dependent upon the concentration of the drug and the concentration of "peptone" in the media. "Peptone," in this connection, they define as any product of protein digestion, whether artificially elaborated or arising through the operation of natural enzymatic processes.

They demonstrated experimentally in cultures that as little as 0.01 mg. of peptone per cubic centimeter definitely affects the bacterial population curve in that even these small traces had a definite inhibitory influence upon the action of sulfanilamide. They suggest as a tentative explanation of the action of sulfanilamide that the drug interferes with the ability of susceptible bacteria to utilize for food the protein-split products in serum, and that death then occurs through starvation and autolysis. The inhibitory effect of peptone, they suggest, arises from the fact that it supplies an excess of easily assimilable nitrogenous material which enables the bacteria to overcome the bacteriostatic action of sulfanilamide.

Another recent contribution to the mechanism of sulfanilamide action is that of Green²¹ based upon extensive experiments, mainly with *Br. abortus*. Green suggests that the action of sulfanilamide depends upon three factors:

(a) The rate of proliferation in the initial growth phase. If, in a fluid medium, the bacterial density reaches a certain level, which Green calls "critical," there is an accumulation in the medium of some antisulfanilamide factor sufficient to prevent further sulfanilamide effect. Possibly Green's "antisulfanilamide factor" may be the "peptone" of Lockwood and Lynch.

(b) The rate at which sulfanilamide reaches a concentration in the bacterial cell. The rate, he says, depends mainly upon the concentration of sulfanilamide in the medium, possibly in part on the nature of the bacterial envelope.

(c) The permeability of the capsule to the antisulfanilamide factor which is either released or formed during autolysis of the dead or dying bacterial cell and which diffuses into the surrounding medium. He suggests that the rate of such release and diffusion may be related to the chemical nature of the capsule and/or cell membrane peculiar to the bacterial species.

Green calls his antisulfanilamide factor the "P" or proliferation factor and suggests that it stimulates the same enzyme reaction in the bacterial cell which sulfanilamide inhibits, the reaction being probably fundamental in the metabolism of most, if not all, bacteria, but not in that of other living cells. This "P" factor is not species specific in source or action. He believes it probable that the sensitivity to sulfanilamide of any bacterial strain is conditioned by the rate at which the "P" factor is liberated by the autolyzing organism and that, under certain conditions, a balance may exist between sulfanilamide and the "P" factor when the organism, though still living, cannot reproduce.

This hypothesis may explain the clinical observation that infections apparently under control after the administration of sulfanilamide are exacerbated when the administration of the drug is stopped too soon.

Also of clinical importance is Green's observation that sulfanilamide-resistant strains may develop and that, under certain conditions, sulfanilamide may even act as a stimulant of bacterial growth because of the elaboration of the "P" factor.

REFERENCES

1. The Genuine Works of Hippocrates, Translated by Francis Adams, L.L.D. (London, 1859), Sydenham Society 1: 324. Quoted by Major, R. H.: *Classic Descriptions of Disease*, ed. 2, Springfield, Ill., 1939, Charles C. Thomas, p. 612.
2. The Extant Works of Aretaeus the Cappodocian, Edited and translated by Francis Adams (London, Sydenham Society, p. 261, 1856. Quoted by Major, R. H.: *Classic Descriptions of Disease*, ed. 2, Springfield, Ill., 1939, Charles C. Thomas, p. 613.
3. Whitby, L. E. H.: *Chemotherapy of Pneumococcal and Other Infections With 2-(p-Aminobenzenesulphonamido) Pyridine*, *Lancet* 1: 1210, 1938.
4. Evans, G. M., and Gaisford, W. F.: *Treatment of Pneumonia With 2-(p-Aminobenzenesulphonamido) Pyridine*, *Lancet* 2: 14, 1938.
5. Bullowa, J. G. M., Osgood, E. E., Bukantz, S. C., and Brownlee, I. E.: *Effect of Sulfapyridine Alone and With Serum on Pneumococci Pneumonia and on Pneumococcus-Infected Marrow Cultures*, *Am. J. M. Sc.* 199: 364, 1940.
6. St. George, A. V., Kraetzer, A. F., and Magee, W.: *Treatment of Pneumonia With 2-(p-Aminobenzenesulphamido) Pyridine*, *Am. J. Clin. Path.* 10: 97, 1940.
7. Doughty, J. F.: *Sulfanilamide Cyanosis Relieved by Nicotinic Acid*, *J. A. M. A.* 114: 754, 1940.
8. McGinty, A. P., Lewis, G. T., and Holtzelaw, M. R.: *Symptoms Occurring With Sulfanilamide Relieved by Nicotinic Acid*, *J. M. A. Georgia* 28: 54, 1939; *Abstr. J. A. M. A.* 112: 1996, 1939.
9. Haviland, J. W., and Blake, F. G.: *Parenteral Administration of Sulfapyridine*, *Am. J. M. Sc.* 199: 385, 1940.
10. Graham, D., Warner, W. P., Dauphinee, J. A., and Dickson, R. C.: *Treatment of Pneumococcal Pneumonia, With Dagenan (M. & B. 693)*, *Canad. M. A. J.* 40: 325, 1939.
11. Powell, H. M., and Chen, K. K.: *Action of Sulfapyridine (2-Sulfanilylamino)pyridine*, *J. Pharmacol. & Exper. Therap.* 67: 79, 1939.
12. Seudi, J. V., Ratish, H. D., and Bullowa, J. G. M.: *Increased Glycuronate Excretion Following Administration of Sulfapyridine*, *Science* 89: 516, 1939.
13. MacLeod, C. M.: *Chemotherapy of Pneumococcal Pneumonia*, *J. A. M. A.* 113: 1405, 1939.
14. Long, H. P., and Wood, W. B., Jr.: *Observations Upon Experimental and Clinical Use of Sulfapyridine*, *Ann. Int. Med.* 13: 487, 1939.
15. Toomey, J. A.: *Urinary Concretions and Sulfapyridine*, *J. A. M. A.* 113: 250, 1939.
16. Antopol, W., and Robinson, H.: *Urolithiasis and Renal Pathology After Oral Administration of 2 (Sulfanilylamino) Pyridine (Sulfapyridine)*, *Proc. Soc. Exper. Biol. & Med.* 40: 428, 1939.
17. Gross, B., and Cooper, F. B.: *Toxicity of M. & B. 693*, *Brit. M. J.* 1: 1058, 1939.
18. Gross, B., Cooper, F. B., and Lewis, M. L.: *Urinary Concretions Caused by Sulfapyridine*, *Proc. Soc. Exper. Biol. & Med.* 40: 448, 1939.
19. Gross, B., Cooper, F. B., and Lewis, M. L.: *Fate of Urinary Calculi Caused by Administration of Sulfapyridine*, *Urol. & Cutan. Rev.* 43: 439, 1939.
20. Gross, B., Cooper, F. B., and Lewis, M. L.: *Urinary Calculi Caused by Sulfapyridine*, *Ibid.* 43: 299, 1939.
21. Loc. cit. Reference 14.
22. Tsao, Y. F., McCracken, M. E., Chen, J., Kuo, P. T., and Dale, C. L.: *Renal Complication in Sulfapyridine Therapy*, *J. A. M. A.* 113: 1316, 1939.
23. Hades, H. L., Stifter, W. C., Jr., Walker, E., McCarty, M., and Shirley, R. G.: *Use of Sulfapyridine in Primary Pneumococcal Pneumonia and in Pneumococcal Pneumonia With Measles*, *J. Pediat.* 14: 417, 1939.
24. Barnett, H. L., Hartmann, A. F., Perley, A. M., and Ruhoff, M. B.: *Treatment of Pneumococcal Infections in Infants and Children With Sulfapyridine*, *J. A. M. A.* 112: 518, 1939.
25. Loc. cit. Reference 18.
26. Dolgopel, V. B., and Hobart, H. M.: *Granulocytopenia in Sulfapyridine Therapy*, *J. A. M. A.* 113: 1012, 1939.
27. David, J. M., and Chesner, C.: *A Fatal Case of Hemolytic Anemia and Nephrotic Uremia Following Sulfapyridine Administration*, *Am. J. M. Sc.* 199: 380, 1940.
28. Loc. cit. Reference 5.
29. Lockwood, J. S., and Lynch, H. M.: *Studies on the Mechanism of the Action of Sulfanilamide. IV. The Influence of Proteolytic Products on the Effectiveness of Sulfanilamide*, *J. A. M. A.* 114: 936, 1940.
30. Green, H. N.: *The Mode of Action of Sulfanilamide With Special Reference to a Bacterial Growth-Stimulating Factor ("P" Factor) Obtained From Br. abortus and Other Bacteria*, *Brit. J. Exper. Path.* 21: 38, 1940.

CLINICAL AND EXPERIMENTAL

HYPERTENSION IN RELATION TO HEIGHT*

ITS VARIATION WITH BODY BUILD AND OBESITY

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NO RIGIDLY controlled statistical study on a large representative sample has ever established a correlation between height and blood pressure. This question is apparently settled in medical literature, since the majority of workers in the field have not been able to show any such relationship. Alvarez and Stanley,¹ most often quoted in this connection, say, "There is no correlation with height." Wunderlich,² in a study of German students, found "no correlation with stature." Reed and Love³ on a large group of United States Army Officers modified the statement somewhat and said the blood pressure was "but slightly, if at all, correlated with height." Stocks and Karn⁴ stressed the fact that even in early life, with other factors held constant, systolic pressure is not correlated with height.

Hunter⁵ found a slight increase in pressure with height, as did the Joint Committee on Mortality.⁶ This increase was greatest at an early age and least later in life. It must be remembered, however, that in many insurance studies height is obtained merely by questioning the prospective policy holder. Rueker,⁷ in a review of the literature, stated that at a very early age, before puberty, blood pressure is usually said to be correlated to length of the body, but Richey⁸ found that even this correlation disappeared when weight was held constant.

In contrast to these statistical studies the clinical impression among physicians is that there is a relation between hypertension and short stature. Fishberg,⁹ and other writers, characterized the hypertensive person as *short and stocky*. The fact that many men say "short and stocky" implies a height relation. We, therefore, find that the statistical evidence shows no correlation between stature and blood pressure, while clinicians are impressed that hypertension is found more commonly among short persons.

In our study of 10,883 persons we found a positive correlation of height to blood pressure in the different build and weight groups, a finding contrary to the general clinical impression.

*From the Department of Medicine, Woodlawn Hospital and Northwestern University.
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STATISTICAL ANALYSIS

The distribution of 10,883 persons according to age, weight, sex, etc., details of examination, and careful estimation of blood pressure, build, and obesity are all presented in previous papers.^{10, 11} In general, the persons were examined in the nude. Weight was recorded on a beam scale; maximum height was obtained to a tenth of an inch; chest circumference was measured with a steel tape slightly above the nipple line during quiet breathing. Blood pressure was recorded with a mercury manometer on the left arm of the seated subject. The group was representative of the adult population between the ages of 20 and 70* years.

DISTRIBUTION OF HEIGHT

The average height of the men was 68.3 inches (68.2 inches when standardized to the United States population of 1934), with a standard range of 65 to 71 inches (Table I). This average is only a few tenths of an inch lower than that found by most other studies on the distribution of height. The Medico-Actuarial Investigation¹² found an average height of 68.5 inches. The average height of the women was 63.6 inches, with a standard range from 61 to 66 inches. Other studies on women show substantially the same average height. Dublin¹⁴ found 64.3 inches, while the Medico-Actuarial Mortality Investigation¹³ found 64.2 inches.

TABLE I
HEIGHT DISTRIBUTION OF 7,478 MEN AND 3,405 WOMEN

	MEN	WOMEN
Mean height	68.3 \pm 0.07	63.6 \pm 0.06
Median height	68.4	63.6
Mode height	68.6	63.6
Standard deviation	2.8	2.5
Standard range	65-71	61-66
Percentage under 60 inches	-1	7
Percentage 60-65 inches	11	66
Percentage 65-70 inches	61	26
Percentage 70 inches and over	27	-1

TABLE II
MEAN HEIGHT IN INCHES OF 7,478 MEN AND 3,405 WOMEN IN EACH DECADE

AGE	20-29	30-39	40-49	50-59	60-69	70 AND OVER
Men	68.7	68.3	68.0	67.8	67.5	67.3
Women	63.9	63.7	63.4	63.3	62.6	61.6

With an increase in age there is a slight decrease in the average height of a large group of persons (Table II). The greatest average height in young men was 68.7 inches; at 40 years of age it decreased to 68.0 inches, and at 70 years it was 67.3 inches. The group of women showed the same tendency. At 20 years of age the mean height was 63.9 inches; at 40 years it was 63.4 inches, and over

*For detailed description see: Robinson, S. C., and Bruce, M.: Range of Normal Blood Pressure, Arch. Int. Med. 61: 402, 1939.

70 years of age it was 61.6 inches. In both men and women the median and modal heights follow a similar age curve. This diminution of height represents a physiologic process of shrinking that most persons experience due to compression of the intervertebral disks, and also to postural changes.

But much more interesting than this decrease of the average stature of man with years is the tendency for the number of tall men and women to decrease after the third decade. Whereas at the age of 20, 5.7 per cent of the men are over 72 inches in height, after 60 years less than 1 per cent of the men are very tall. While 4.6 per cent of women are taller than 67 inches in the third decade, less than 1 per cent of women over 60 years of age are very tall. This is a very striking and statistically significant finding. We do not have an adequate explanation for this phenomenon, but shall discuss it later in terms of hypertension in the tall person. Increased mortality among tall persons is one of the explanations that must be seriously considered. On the other hand, the age incidence of short men and women increases as the group gets older. There are comparatively more short men in the older age groups than in the younger age groups.

TABLE III
INCIDENCE OF TALL MEN AND WOMEN AT VARIOUS AGES

AGE	20-29	30-39	40-49	50-59	60-69	70 AND OVER
Percentage of men under 63 inches	1.1	3.1	4.1	3.7	4.1	5.5
Percentage of men over 70 inches	19.5	14.5	13.1	11.1	8.8	2.8
Percentage of men over 72 inches	5.7	3.7	3.5	2.3	0.9	0
Percentage of women under 59 inches	1.0	2.6	4.4	5.0	6.6	20.0
Percentage of women over 65 inches	19.7	19.2	14.6	15.5	5.7	0
Percentage of women over 67 inches	4.6	3.5	2.3	2.6	0.9	0

RELATION OF HEIGHT TO BLOOD PRESSURE

If the body builds and weights of individuals are mixed, as they are in the chance distribution of any sample, height shows a slight correlation to blood pressure* (Table IV). The mean blood pressure of the men shows the short group to have slightly higher systolic and diastolic pressures than the tall groups (Fig 1). The short women have higher mean systolic and diastolic pressures than the tall women.

In order to study the possible effect of height on blood pressure, we divided the group into three height classifications: the very short, the medium, and the

*The correlation coefficients for the relation of height and blood pressure are as follows:

Men		Systolic Blood Pressure	Women	
r hs	$= -0.037 \pm 0.012$		r hs	$= -0.095 \pm 0.017$
η hs	$= 0.061$		η hs	$= 0.121$
ξ hs	$= 0.003 \pm 0.003$		ξ hs	$= 0.007 \pm 0.0003$
Men		Diastolic Blood Pressure	Women	
r hs	$= -0.002 \pm 0.012$		r hs	$= -0.030 \pm 0.017$
η hs	$= 0.051$		η hs	$= 0.072$
ξ hs	$= 0.001 \pm 0.0007$		ξ hs	$= 0.004 \pm 0.002$

Thus each correlation is a linear negative one and so small that without further careful investigation it would seem that height is not at all significant as a correlation to blood pressure.

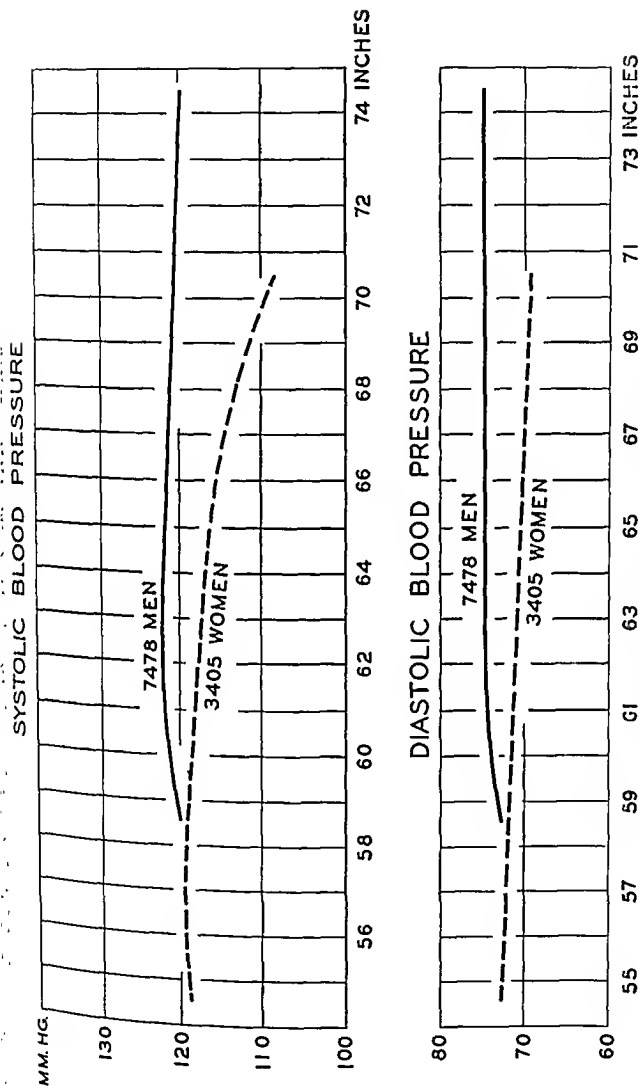


Fig. 1.—The influence of height on blood pressure. In the total group, with various builds and weights indiscriminately mixed, the average blood pressure is slightly higher in short men and women than in tall.

RELATION OF HEIGHT TO BLOOD PRESSURE

TABLE IV

HEIGHT IN INCHES	UNDER 63	63-64	65-66	67-68	69-70	71-72	73-74	75 AND OVER	ALL HEIGHTS	CORRELATION DATA				
										MEAN	MODE	HEIGHT	BLOOD PRESSURE	
Systolic, 7,478 Men														
No. of persons	237	709	1,541	2,099	1,814	799	231	48	7,478	Mean	68.5	68.2 ± 0.03	121.0 ± 0.20	
Mean pressure	120	123	122	121	120	120	120	120	121	Std. dev.	2.83	2.83	17.35	
Median pressure	117	121	120	119	119	120	118	119	119	Skewness	-0.11	-0.11	0.29	
Modal pressure	112	115	117	115	117	119	113	119	116	Coef. var.	4.15	4.15	14.34	
% under 110 mm.	28	22	23	21	22	22	19	23	22	Coef. corr.	r =	-0.037 ± 0.012		
% 140 mm. and over	14	15	13	10	9	8	10	8	11	Corr. ratio	η =	0.061		
										Test lin.	ξ =	0.003 ± 0.0003		
Diastolic, 7,478 Men														
No. of persons	237	709	1,541	2,099	1,814	799	231	48	7,478	Mean	68.5	68.2 ± 0.03	74.4 ± 0.12	
Mean pressure	73	75	75	74	74	74	75	74	74	Std. dev.	2.83	2.83	10.44	
Median pressure	72	73	74	73	73	73	74	73	73	Skewness	-0.11	-0.11	0.32	
Modal pressure	71	70	71	71	72	71	72	71	71	Coef. var.	4.15	4.15	14.03	
% under 70 mm.	37	30	29	30	30	32	24	33	30	Coef. corr.	r =	-0.002 ± 0.012		
% 90 mm. and over	9	9	8	7	6	6	6	4	7	Corr. ratio	η =	0.051		
										Test lin.	ξ =	0.001 ± 0.0007		
CORRELATION DATA														
71 AND ALL OVER HEIGHTS														
Systolic, 3,404 Women														
No. of persons	109	392	887	1,055	674	246	38	3	3,404	Mean	63.6	63.6 ± 0.04	117.0 ± 0.35	
Mean pressure	124	121	117	117	116	112	112	106	117	Mode	63.6	63.6	112.4	
Median pressure	114	115	113	114	113	112	109	105	113	Std. dev.	2.45	2.45	20.22	
Modal pressure	112	103	107	107	107	112	112	103	112	Skewness	-	-	0.23	
% under 110 mm.	37	38	39	38	40	42	55	67	39	Coef. var.	3.85	3.85	17.28	
% 140 mm. and over	25	16	10	9	8	4	2	0	10	Coef. corr.	r =	-0.095 ± 0.017		
										Corr. ratio	η =	0.127		
										Test lin.	ξ =	0.007 ± 0.003		
CORRELATION DATA														
71 AND ALL OVER HEIGHTS														
Diastolic, 3,404 Women														
No. of persons	109	392	887	1,055	674	246	38	3	3,404	Mean	63.6	63.6 ± 0.04	71.0 ± 0.20	
Mean pressure	72	72	71	71	71	70	73	64	71	Mode	63.6	63.6	70.1	
Median pressure	71	71	71	71	71	71	71	65	71	Std. dev.	2.45	2.45	11.58	
Modal pressure	69	69	70	71	70	72	66	70	70	Skewness	-	-	0.09	
% under 70 mm.	40	45	46	46	47	47	45	47	47	Coef. var.	3.85	3.85	10.31	
% 90 mm. and over	10	10	10	10	10	10	10	10	10	Coef. corr.	r =	-0.049 ± 0.017		
										Corr. ratio	η =	0.025		
										Test lin.	ξ =	0.003 ± 0.0003		

very tall.* Because this division is used only for comparisons between the extremes, the arbitrary nature of the division makes little difference. If the divisions had been made either an inch higher or an inch lower, the results would have been essentially the same. It would be well, however, to establish a standard division in future statistical work.

TABLE V
MEAN SYSTOLIC PRESSURE IN VARIOUS HEIGHT CLASSIFICATIONS

HEIGHT	7,478 MEN	3,405 WOMEN
Short	121.9 \pm 0.90	117.6 \pm 0.82
Medium	120.0 \pm 0.49	115.1 \pm 0.70
Tall	119.7 \pm 0.56	114.1 \pm 1.01

TABLE VI
MEAN DIASTOLIC PRESSURE IN VARIOUS HEIGHT CLASSIFICATIONS

HEIGHT	7,478 MEN	3,405 WOMEN
Short	75.3 \pm 0.51	70.6 \pm 0.39
Medium	74.4 \pm 0.29	70.1 \pm 0.41
Tall	73.7 \pm 0.34	70.1 \pm 0.66

The blood pressure in these three height groups shows an interesting comparison (Table V). There is a 2.2 mm. difference in systolic pressure between short and tall men and 3.5 between short and tall women. These differences are statistically significant.† It is improbable that a purely chance distribution would cause such a difference.

The situation in diastolic pressure is similar to that in systolic pressure (Table VI). The difference of 1.6 mm. between the short and tall men is significant of an actual difference not due to chance errors in distribution.‡ The

*Because the differences between short and tall individuals were unexpectedly worthy of consideration, the two extreme height groups were augmented by a random selection from our files of 915 additional tall and short individuals.

Men

359 (plus 157) short	= under 66 inches
1,212 medium	= 66 to 70 inches
290 (plus 534) tall	= 71 inches and over

Women

734 (plus 208) short	= under 63 inches
767 medium	= 63 to 65 inches
296 (plus 16) tall	= 66 inches and over

These augmented groups were not used in Tables I and II, but were used only for the blood pressure comparison between short and tall persons so as not to affect the random character of the total sample. All discussions concerning age, height, and obesity (ponderal index) distributions are based on figures from the original sample of 7,478 men and 3,405 women. The discussion of blood pressures is based on a portion of this sample, 1,861 men and 1,797 women. The blood pressures of the original and the augmented groups are practically identical, which supports the genuine character of the results.

Men

Short	= 65 inches and under
Medium	= 66 to 70 inches
Tall	= 71 inches and over

Women

Short	= 62 inches and under
Medium	= 63 to 65 inches
Tall	= 66 inches and over

†The standard difference between the pressures of the short and tall men shows that to be least 2.5 mm. Because the actual differences are slightly greater than this standard difference, it is improbable that the difference is due to a pure chance distortion of the distribution.

‡The standard difference shows that to be significant the difference between the diastolic pressure of short and tall men must be at least 1.22 mm. For women the difference in diastolic pressure must be at least 1.52 mm.

difference of 0.5 mm. for women is not significant. Thus, in our total amalgam groups short men have slightly higher average systolic and diastolic pressures than tall men, and short women have slightly higher average systolic pressure than tall women.

The incidence of low and high pressures in short and tall men and women brings out more significantly the extent of these differences in the two extreme height groups. Twenty-five per cent of the short men have low systolic blood pressures, and 22 per cent of the tall men have systolic pressures under 110 mm. Forty-one per cent of short and tall women have systolic pressures under 110 mm. On the other hand, both men and women show a higher incidence of high pressures in the short groups than in the tall. Fourteen per cent of short men have high systolic pressures of 140 mm. and over, while only 9 per cent of tall men show these same high pressures. Twelve per cent of short women have a high systolic pressure, while only 5 per cent of tall women have pressures of 140 mm. and over.

Diastolic pressure shows a somewhat similar incidence variation. Again there is not much difference between the incidence of low pressures; 27 per cent of short men have diastolic pressures under 70 mm., while 29 per cent of tall men have these same low pressures. Fifty-one per cent of short women have low diastolic pressures, while 47 per cent of tall women have diastolic pressures under 70 mm. The incidence of high diastolic pressures shows significant differences between short and tall persons. Short men show an incidence of 8 per cent diastolic pressures of 90 mm. and over, while tall men show considerably fewer, only 5 per cent, high diastolic pressures. Short women show an incidence of 8 per cent and tall women 5 per cent high diastolic pressures.

It is thus seen that the mean as well as the distribution of low and high pressures both prove that the short men or women have more hypertension than their tall brothers or sisters. Offhand, without any further statistical breakdowns of the height groups studied, one might be led to believe, first, that stature per se exerted a strong influence on blood pressure and, second, that the short person was the one influenced more unfavorably. When the build and weight of each height group are held constant, it will be shown that the former statement is correct and the latter is false.

The data just presented were of the totals of each height group. To arrive at a true correlation of height to blood pressure it is necessary to separate our groups according to age, weight, build, and other factors that are known to be related to blood pressure.¹⁰⁻¹² If the short group has a larger number of persons that are fat and stocky, and if obesity and stockiness are related to hypertension, then the short group will show more hypertension but it will not necessarily be due to stature. To avoid such an error we must correct for these disturbing factors. This we do by holding the build and the weight factors constant in the different height groups.

RELATIONSHIP OF HEIGHT TO BLOOD PRESSURE IN SPECIFIC BUILD GROUPS

It is generally recognized by most anthropometrists that the gross morphology of mankind can be divided roughly into two contrasting build types,

the thin and the broad. The frame of the broad build person can be differentiated from the very narrow type of build by the chest/height ratio. This figure is obtained by dividing the chest circumference in inches by the height in inches. It is to be noted that the measure of weight is not used at all to arrive at this ratio. The simplicity of the index is its main asset and can be pictured easily by the rule-of-thumb statement that the chest circumference should be one-half the height, or a chest/height ratio of 0.50.

When the group is broken down into linear, intermediate, and lateral build classifications, slight differences are apparent (Table VII). Neither the linear nor the lateral build men show a statistically significant difference in mean or modal systolic or diastolic pressure between the short and tall classifications.

TABLE VII

COMPARISON OF MEAN AND MODAL PRESSURES OF 2,552 MEN AT VARIOUS HEIGHTS IN SPECIFIC BUILD GROUPS

PRESSURE			LINEAR BUILD (SLENDER)	INTERMEDIATE BUILD	LATERAL BUILD (BROAD)
Systolic	Short men	Mean	113.8	120.7	127.5
		Mode	107.5	112.7*	122.7*
	Medium men	Mean	113.7	120.5	125.3
		Mode	112.1*	110.3	119.0
	Tall men	Mean	116.8	120.8	130.6
		Mode	110.8	113.0	121.3*
Diastolic	Short men	Mean	70.5	74.2	79.6
		Mode	70.8	70.6	80.5
	Medium men	Mean	70.4	74.8	77.8
		Mode	72.5	70.6	81.1
	Tall men	Mean	71.1	74.9	81.5
		Mode	72.3	70.7	80.0

*Estimated mode.

TABLE VIII

COMPARISON OF MEAN AND MODAL PRESSURES OF 2,021 WOMEN AT VARIOUS HEIGHTS IN SPECIFIC BUILD GROUPS

PRESSURE			LINEAR BUILD (SLENDER)	INTERMEDIATE BUILD	LATERAL BUILD (BROAD)
Systolic	Short women	Mean	109.6	115.4	127.9
		Mode	107.2	105.2	113.0*
	Medium women	Mean	110.1	116.0	128.4
		Mode	106.5	112.1*	116.4
	Tall women	Mean	109.1	118.1	134.0
		Mode	109.7	112.7*	122.0
Diastolic	Short women	Mean	66.8	69.7	75.9
		Mode	62.3	70.0	71.1
	Medium women	Mean	66.9	70.8	77.0
		Mode	63.6	71.4	70.4
	Tall women	Mean	66.4	72.6	84.0
		Mode	63.1	71.1	79.5

*Estimated mode.

The short linear women show the same mean and modal pressures that are found in the tall linear women. However, the intermediate build women show slightly higher pressures if they are tall than if they are short (Table VIII). This difference is most marked among the women of lateral build. But the maximum difference of about 8 mm. between short and tall in the lateral build is not statistically significant. Notice that the small differences which

do appear, while not statistically significant, are reversed from what was found in the total group. Tall persons show a greater hazard of hypertension than short persons in specific build groups.

Had we stopped at this point in our statistical survey of the relation of height to blood pressure even in the build breakdowns, we could not show any statistical correlation. Other studies, thus far, have relied solely upon the mean and mode, and hence, were unable to demonstrate any correlation.

More important than the mean or modal pressure is a study of the percentage distribution of low and high pressures in each build group of short and tall men and women. The tall linear men show fewer low pressures and more high pressures than short linear men (Table IX).

TABLE IX

COMPARISON OF THE INCIDENCE OF LOW AND HIGH SYSTOLIC PRESSURES IN 516 SHORT AND 824 TALL MEN

PRESSURE		LINEAR BUILD % (SLENDER)	INTERMEDIATE BUILD %	LATERAL BUILD % (BROAD)
Low	Short men	47*	26	16
	Tall men	30	19	13
High	Short men	3*	14	18
	Tall men	5	10	29

*Small distribution—less than 50 short linear men.

TABLE X

COMPARISON OF THE INCIDENCE OF LOW AND HIGH DIASTOLIC PRESSURES IN 516 SHORT AND 824 TALL MEN

PRESSURE		LINEAR BUILD % (SLENDER)	INTERMEDIATE BUILD %	LATERAL BUILD % (BROAD)
Low	Short men	47*	30	16
	Tall men	39	24	13
High	Short men	3*	6	16
	Tall men	1	6	23

*Small distribution—less than 50 short linear men.

The same relation is apparent in diastolic pressure (Table X). Although there is a smaller percentage of high pressure among tall linear men than among short linear men, the distribution in this group is small.

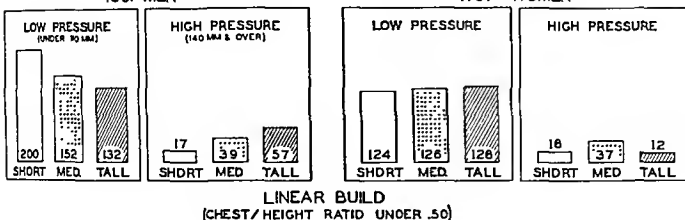
More important are the comparative incidences among lateral or broad build men. Tall men show a greater incidence of hypertension, both systolic and diastolic, than short men, and less low pressure. Whereas 16 per cent of short lateral men have low systolic and diastolic pressures, only 13 per cent of tall lateral men have these same low pressures. Only 18 per cent of short lateral men have systolic hypertension, whereas 29 per cent of tall lateral men have high systolic pressures. Sixteen per cent of short lateral men have diastolic hypertension compared with 23 per cent high pressures among tall lateral men.

The women show a similar relation within their height classifications (Table XI). Tall linear women show less low pressure and more high pressure, both systolic and diastolic, than short women. Among the lateral build women this is more marked. Only 12 per cent of tall lateral women have low systolic

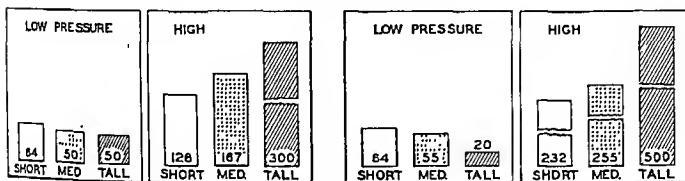
PERCENT ACTUAL TO EXPECTED RATIO

1861 MEN

1797 WOMEN



LINEAR BUILD
(CHEST/HEIGHT RATIO UNDER .50)



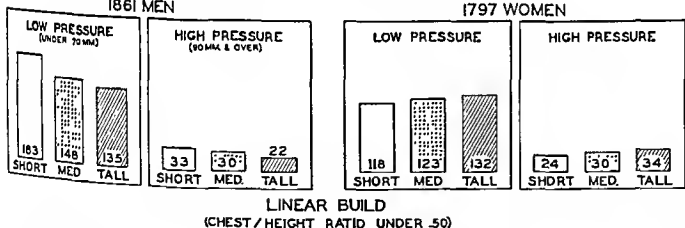
LATERAL BUILD
(CHEST/HEIGHT RATIO 59 & OVER)

Fig. 2.—The influence of height on systolic blood pressure in specific build groups. The actual to expected ratio shows that in either linear or lateral build groups the tall men will show fewer low systolic pressures and more high systolic pressures than the short men. The same is true for lateral build, though not for linear build, women.

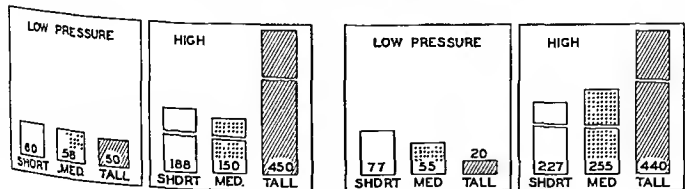
PERCENT ACTUAL TO EXPECTED RATIO

1861 MEN

1797 WOMEN



LINEAR BUILD
(CHEST/HEIGHT RATIO UNDER .50)



LATERAL BUILD
(CHEST/HEIGHT RATIO 59 & OVER)

Fig. 3.—The influence of height on diastolic blood pressure in specific build groups. The actual to expected ratio shows that in the lateral or broad build group tall men and women will show fewer low diastolic pressures and more high diastolic pressures than the short men and women.

pressures, while fully 26 per cent of short lateral women have these same low pressures, under 110 mm. Only 12 per cent of tall lateral women have low diastolic pressures, while 36 per cent of short lateral women have these same low pressures (Table XII).

TABLE XI

COMPARISON OF THE INCIDENCE OF LOW AND HIGH SYSTOLIC PRESSURES IN 942 SHORT AND 312 TALL WOMEN

PRESSURE		LINEAR BUILD %	INTERMEDIATE	LATERAL BUILD %
		(SLENDER)	BUILD %	(BROAD)
Low	Short women	53	44	26
	Tall women	52	32	12
High	Short women	3	9	29
	Tall women	0.6	8	30

TABLE XII

COMPARISON OF THE INCIDENCE OF LOW AND HIGH DIASTOLIC PRESSURES IN 942 SHORT AND 312 TALL WOMEN

PRESSURE		LINEAR BUILD %	INTERMEDIATE	LATERAL BUILD %
		(SLENDER)	BUILD %	(BROAD)
Low	Short women	60	51	36
	Tall women	62	35	12
High	Short women	2	6	18
	Tall women	2	8	24

The lateral build women show the same trend as men in respect to high pressure, although the difference in systolic pressure between short and tall women is not significant. However, 24 per cent of tall women have diastolic hypertension as compared with 18 per cent of short women.

It is thus seen that the study of the incidence of low and high pressures in different build groups shows a significant difference between tall and short persons. Generally speaking, the tall individual has a lower incidence of low pressure and a greater incidence of high pressure. This is most marked in the lateral build groups.

The actual to expected ratio* brings out comparative differences in a more illustrative manner and corrects for changes in distribution between the various heights. First, we shall discuss the linear or thin build. By segregating these persons in each height group and then comparing the actual to expected ratio of low and high systolic pressures, we find that the short linear or thin men have one and one-half times as many low systolic pressures, and nearly that many more low diastolic pressures as the tall thin men (Tables XIII and XIV). However, in the case of high systolic pressures the short thin men

*The per cent actual to expected ratio is very simply calculated and is of use in illustrating the relative effect of the extremes of one array upon the dispersion of another correlated array. The technique is used here to show the unusual influence of a broad build on blood pressure. For example, if in any random group of 100 males, 24 are of the linear type and 13 are of the lateral type, and if there is no correlation of build to blood pressure, then the same ratio of 24 linear types to 13 lateral types should hold true in any group of individuals whether they have low, medium, or high pressures. This, however, is not the case. Among a group of 100 hypertensive persons there are actually only 9 linear types to 23 lateral types. This gives a per cent actual to expected incidence of $9/24$, or a ratio of only 38 per cent of expected linear build hypertensive individuals as compared with $23/13$, or a ratio of 177 per cent of expected lateral build hypertensive individuals. Simply stated, this means that among hypertensive persons the lateral build predominates in a ratio of over 4 to 1. When this is done in each height group, the ratios can be compared as they correct for changes in the height-build distribution.

have only one-third the ratio shown for tall thin men (Figs. 2 and 3). The difference is reversed for diastolic pressure. In comparison of the short and tall men of the lateral or broad build groups we also find that the short men have more favorable pressures. There are about two and one-half times as many systolic and diastolic hypertensives among the tall men as among the short lateral men. The ratio for low pressures is also slightly in favor of the short men.

TABLE XIII

PER CENT ACTUAL TO EXPECTED INCIDENCE OF LOW AND HIGH SYSTOLIC PRESSURES AT VARIOUS HEIGHTS IN LINEAR AND LATERAL BUILD MEN AND WOMEN

	LOW PRESSURE*	HIGH PRESSURE		LOW PRESSURE	HIGH PRESSURE
Linear Build* (Slender)					
Short men	200	17	Short women	124	18
Medium Men	152	39	Medium women	126	37
Tall men	132	57	Tall women	128	12
Lateral Build (Broad)					
Short men	64	128	Short women	64	232
Medium men	50	167	Medium women	55	255
Tall men	50	300	Tall women	20	500

*For the purpose of this and other similar charts the terms used are defined as follows:
 Low pressure = Under 110 mm. systolic pressure and 70 mm. diastolic pressure
 High pressure = 140 mm. and over systolic pressure and 90 mm. and over diastolic pressure.
 Linear or slender build = Chest / height ratio under 0.50.
 Lateral or broad build = Chest / height ratio 0.59 and over.

ary and is used only to bring out the difference in the extremes his study finer subdivisions of chest/height ratio are usually type into the other. It does not imply that the intermediate of normal levels is discussed in another publication.

TABLE XIV

PERCENTAGE ACTUAL TO EXPECTED INCIDENCE OF LOW AND HIGH DIASTOLIC PRESSURES AT VARIOUS HEIGHTS IN LINEAR AND LATERAL BUILD MEN AND WOMEN

	LOW PRESSURE	HIGH PRESSURE		LOW PRESSURE	HIGH PRESSURE
Linear Build (Slender)					
Short men	183	33	Short women	118	24
Medium men	148	30	Medium women	123	30
Tall men	135	22	Tall women	132	34
Lateral Build (Broad)					
Short men	60	188	Short women	77	227
Medium men	58	150	Medium women	55	255
Tall men	50	450	Tall women	20	440

This same type of relationship holds for women in the lateral group but not for those in the linear group (Tables XIII and XIV). There is practically no difference in the actual to expected ratio of low and high pressures between the short thin women and the tall thin women. But there are twice as many systolic and diastolic hypertensive persons in the tall lateral as there are in short lateral women and less than one-third the low systolic and diastolic pressures.

In both linear and lateral build groups tall men have a far stronger tendency to develop hypertension and less tendency to develop low pressure

than their shorter brothers. The same holds true for women of the lateral build. This same tendency is reflected in the actual to expected ratio of low to high pressures within a specific build-height group. There will be a ratio of 12 low pressures to 1 high systolic pressure among short linear men, almost 4 low to 1 high among medium height linear men, and only a little more than 2 low to 1 high systolic pressure among tall linear men. This does not hold for diastolic pressure among linear men or for systolic or diastolic pressure among thin women. However, in the lateral build group, an unusual difference was found in both systolic and diastolic pressures among both men and women. Among lateral build persons the ratio is reversed. The lateral build person of any stature is always more susceptible to high pressure than to low pressure, but the tall lateral person carries the greatest hazard of all. Among short lateral men there will be only 2 high systolic pressures to 1 low systolic pressure. Medium height men show a ratio of more than 3 high systolic pressures to 1 low systolic pressure, while tall lateral men show a ratio of 6 high systolic pressures to 1 low systolic pressure. The women show a similar pattern. Short lateral women have almost 4 high systolic pressures to 1 low systolic pressure; medium height women show a ratio of almost 5 high systolic pressures to 1 low systolic pressure; and tall women show a ratio of 25 to 1. In other words, high pressure is far more common among tall lateral persons than among short lateral persons.

Diastolic pressure in lateral build shows the same trend. Among short lateral men there will be a ratio of 3 to 1, and among tall lateral men there will be a 5 to 1 chance of having a high diastolic pressure. Short lateral women show only a 3 to 1 ratio of high to low diastolic pressures, and tall lateral women show 22 high pressures to 1 low pressure. This extreme difference in the tall systolic and diastolic is erratic because of the small number of persons.

Thus it is apparent that for both men and women the tall lateral or broad person showed a greater predilection for systolic and diastolic hypertension than the short lateral person, and a lesser tendency to low pressure.

SUMMARY OF RELATION OF HEIGHT TO BLOOD PRESSURE IN SPECIFIC BUILD GROUPS

The summary of the relation of height to blood pressure in specific builds is brought out in the explanation of an apparent contradiction. We have shown that in our total group of 10,883 men and women the short persons had a higher mean pressure than the tall. On the other hand, after a study of the incidence of low and high pressures and the actual to expected ratio was completed in different build groups, we found that the tall individual was more susceptible to hypertension, especially if he was of the lateral build type. The increase in blood pressure of the tall person over the short *lateral* person is much greater than the increase of the mean pressure of the short person over the tall person in the total group. This is apparently a contradiction and must be explained. The explanation is to be found in the comparative distribution of build types in short and tall persons. In another paper it was shown that any lateral build person is more susceptible to hypertension than the linear type. Roughly, about three times as many lateral persons as linear persons are hypertensive. It can be seen that if there are more lateral builds among short men we would expect more

hypertension in the short group. This is precisely what we found. Twenty-four per cent of the short men are of the lateral build, while only 5 per cent of the tall men have a lateral build. The same is true for women. Thus in any sample of the population there will be far more short persons than tall lateral persons (Fig. 4). Although the tall lateral person is more susceptible to hypertension than the short lateral person, this increased susceptibility is more than offset by the greater number of short lateral persons in the total group. Hence, all short men together show a higher mean pressure than all tall men.

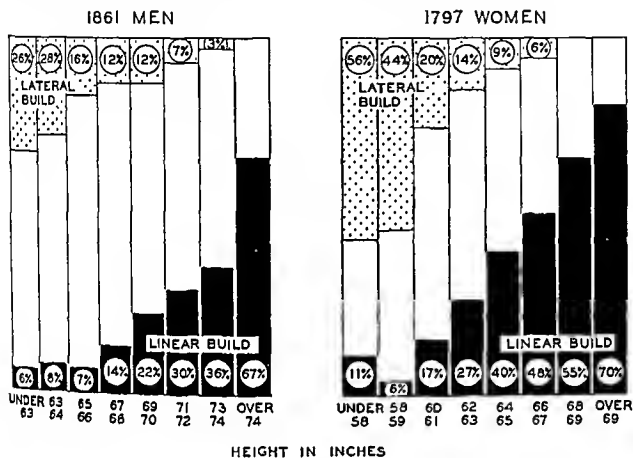


Fig. 4.—The distribution of linear and lateral build at different heights. Lateral or broad build is most frequently found in short men and women, and linear or thin build is most frequent among tall men and women. Blank sections indicate the intermediate build.

The clinical impression that the hypertensive individual is a short stocky man is therefore correct. The physician will see a far larger number of short stocky hypertensive persons and very few tall broad hypertensive ones merely because of the natural distribution of the lateral build in these two height groups. And yet the careful investigator must be aware that the tall broad individual is more susceptible to hypertension than any other person.

RELATION OF HEIGHT TO BLOOD PRESSURE IN SPECIFIC WEIGHT GROUPS

When the short, medium, and tall men and women are divided according to body weight groups, certain variations are important. The weight breakdown is done with the aid of the ponderal index, an index of relative obesity, the weight divided by the height. A low index (under 2.0) denotes a lightweight person, and a high index (2.5 and over) denotes an obese person. In a large sample of persons obesity is positively correlated to an increased blood pressure.

When the mean and modal pressures are compared in the various height-weight groups, no height difference in blood pressure is noticed in either lightweight or middleweight men (Table XV).

TABLE XV

COMPARISON OF MEAN AND MODAL PRESSURES OF 7,478 MEN AT VARIOUS HEIGHTS IN SPECIFIC PONDERAL INDEX GROUPS

PRESSURE			LIGHT- WEIGHT	MIDDLE- WEIGHT	HEAVY- WEIGHT
Systolic	Short men	Mean	117.6	121.5	128.5
		Mode	112.1	113.4	122.6
	Medium men	Mean	115.9	120.4	124.9
		Mode	110.5	114.4	118.0
	Tall men	Mean	117.5	118.3	123.9
		Mode	115.7	114.7	118.2
Diastolic	Short men	Mean	70.9	74.0	79.2
		Mode	71.2	70.7	78.9
	Medium men	Mean	70.5	73.6	78.5
		Mode	72.6	71.2	76.7
	Tall men	Mean	71.9	72.9	77.8
		Mode	70.7	72.0	73.3

TABLE XVI

COMPARISON OF MEAN AND MODAL PRESSURES OF 3,405 WOMEN AT VARIOUS HEIGHTS IN SPECIFIC PONDERAL INDEX GROUPS

PRESSURE			LIGHT- WEIGHT	MIDDLE- WEIGHT	HEAVY- WEIGHT
Systolic	Short women	Mean	111.8	119.8	127.6
		Mode	108.2	106.3	112.9
	Medium women	Mean	111.5	116.8	125.0
		Mode	108.2	112.1	110.9
	Tall women	Mean	109.8	115.6	121.2
		Mode	108.3	110.2	118.2
Diastolic	Short women	Mean	67.5	72.3	76.6
		Mode	63.3	71.1	73.3
	Medium women	Mean	67.6	71.5	75.7
		Mode	63.7	70.6	72.4
	Tall women	Mean	67.2	70.8	75.4
		Mode	67.5	70.5	70.3

TABLE XVII

COMPARISON OF INCIDENCE OF LOW AND HIGH SYSTOLIC PRESSURES IN 1,540 SHORT AND 1,078 TALL MEN (PONDERAL INDEX GROUPS)

PRESSURE		LIGHTWEIGHT PER CENT	MIDDLEWEIGHT PER CENT	HEAVYWEIGHT PER CENT
Low	Short men	35	23	16
	Tall men	24	24	16
High	Short men	9	13	22
	Tall men	3	7	14

TABLE XVIII

COMPARISON OF INCIDENCE OF LOW AND HIGH DIASTOLIC PRESSURES IN 1,540 SHORT AND 1,078 TALL MEN (PONDERAL INDEX GROUPS)

PRESSURE		LIGHTWEIGHT PER CENT	MIDDLEWEIGHT PER CENT	HEAVYWEIGHT PER CENT
Low	Short men	44	31	18
	Tall men	42	34	21
High	Short men	5	7	17
	Tall men	3	3	12

However, in the heavyweight men the tall group shows slightly lower systolic and diastolic pressures. At any height the men show higher pressures as they become heavier. However, the greatest increase is found in the short men where there is an increase of 10.9 mm. in mean pressure between lightweight and heavyweight men. Among tall men there is only a 6.4 mm. difference.

Lightweight, middleweight, and heavyweight women show the same systolic pressures at any height, but the short heavyweight women show a slightly greater diastolic pressure than the tall heavyweight women (Table XVI).

More informative than the mean and mode is a study of the distribution of low and high pressures among tall and short men in specific weight groups. Short men of any weight group show a greater incidence of high systolic and diastolic pressures than tall men (Tables XVII and XVIII).

A similar distribution is noted among women. The short women always show far more high systolic and diastolic pressures at any weight than tall women (Tables XIX and XX).

TABLE XIX

COMPARISON OF INCIDENCE OF LOW AND HIGH SYSTOLIC PRESSURES IN 1,383 SHORT AND 588 TALL WOMEN (PONDERAL INDEX GROUPS)

PRESSURE		LIGHTWEIGHT PER CENT	MIDDLEWEIGHT PER CENT	HEAVYWEIGHT PER CENT
Low	Short women	48	37	23
	Tall women	52	37	27
High	Short women	4	15	26
	Tall women	1	6	9

TABLE XX

COMPARISON OF INCIDENCE OF LOW AND HIGH DIASTOLIC PRESSURES IN 1,383 SHORT AND 588 TALL WOMEN (PONDERAL INDEX GROUPS)

PRESSURE		LIGHTWEIGHT PER CENT	MIDDLEWEIGHT PER CENT	HEAVYWEIGHT PER CENT
Low	Short women	59	40	30
	Tall women	56	47	28
High	Short women	3	7	15
	Tall women	1	5	10

The actual to expected ratio in the weight breakdown is not helpful in revealing the height differences in low and high pressures.

When short and tall persons are compared in specific weight groups, we find a complete reversal of the distribution of high pressures. In all three weight groups, light, middle, and heavy, short men and women have a higher incidence of high systolic and diastolic pressures than tall men and women. In the distribution of low pressures we found no difference between short and tall men and women in any weight group.

DISCUSSION

In our previous studies of 10,883 persons blood pressure was found to be correlated to both body build and obesity. However, since the literature is almost unanimous in stating that height does not affect blood pressure, no study of this

relation was contemplated. Almost accidentally, in a study of body build, it was discovered that the lateral person is most often of short stature. Since hypertension is most common in the lateral build it was reasoned, therefore, that height must show some effect on blood pressure. Accordingly, this study was made of the relation of height to blood pressure, and statistical breakdowns proved our reasoning to be true. The short person had higher mean and modal pressures and a higher incidence of high pressure than the tall person in the total group.

Since we have shown that the lateral build person of any height group is more susceptible to hypertension, it is obvious that the higher mean and modal pressure of the short group is a distortion due to a preponderance of lateral builds among short men. This could be corrected only by holding the build groups constant and then comparing the incidence of high and low pressures between short and tall persons.

When such a study was made of the blood pressure distribution within the various build-height groups, an entirely new and interesting height variation was discovered. The tall lateral person had a higher mean and modal pressure than the short lateral person, and a much greater incidence of hypertension. The tall lateral person had a smaller chance of having a low pressure than did the short lateral person. The actual to expected ratio further confirmed this striking finding. We, therefore, have an apparent contradiction in the influence of height on blood pressure. The true height difference is the greater hazard of hypertension carried by tall broad persons. The reversed finding in the total group showing the short persons to have more hypertension than the tall persons is due to the greater incidence of lateral build in the short group. Thus there is really no contradiction. The influence of build on hypertension is again strongly emphasized.

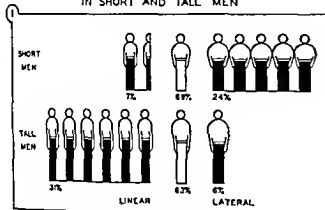
When the group was broken down into specific weight classifications, the short group was shown to have a higher incidence of hypertension than the tall group. This difference held true in all three weight groups. Height, therefore, influences blood pressure in two opposing ways. When the build is held constant, the tall person is more susceptible to hypertension. When the weight is held constant, the short person has the greater incidence of hypertension. How can these two divergent facts be reconciled?

The difference between tall and short in the various build groups is the true constitutional factor influencing blood pressure. This represents the morphologic and biochemical susceptibility to hypertension of the tall broad type, the exact nature of which requires further study. The pressure difference between tall and short men and women in the specific weight groups is dependent upon the greater incidence of lateral build in the short group. For, as we shall show in a subsequent study, obesity is linked to the lateral build. Therefore, we are really measuring to a great degree the lateral build factor when we study blood pressure in the short overweight person.

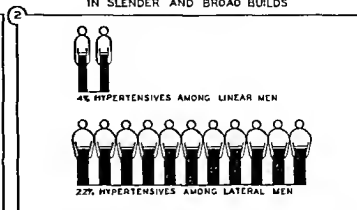
This finding, that hypertension is a greater hazard of the tall lateral person, suggests a comparison with Draper's¹⁵ contention that the women with hyperten-

sion "... are the tallest of all women. . . ." Draper does not make the same statement about men; nevertheless, our study surely confirms Draper's finding and probably holds for both men and women.

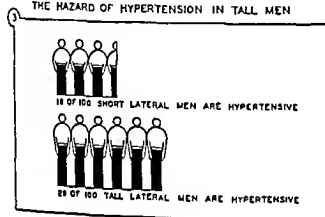
DISTRIBUTION OF BUILD TYPES
IN SHORT AND TALL MEN



INCIDENCE OF HYPERTENSION
IN SLENDER AND BROAD BUILDS



THE HAZARD OF HYPERTENSION IN TALL MEN



THERE ARE MORE SHORT MEN WITH HYPERTENSION

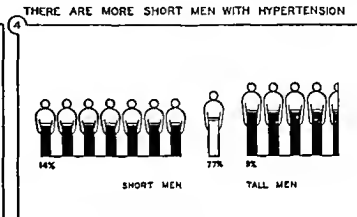


Fig. 5.—This chart shows the relationship of height and build to blood pressure. (1) In a group of 100 men, 11 are tall and 89 are short. (2) In a group of 100 men, 11 are tall and 89 are short. (3) In a group of 100 men, 11 are tall and 89 are short. (4) In a group of 100 men, 11 are tall and 89 are short. (5) In a group of 100 men, 11 are tall and 89 are short. (6) In a group of 100 men, 11 are tall and 89 are short. (7) In a group of 100 men, 11 are tall and 89 are short. (8) In a group of 100 men, 11 are tall and 89 are short. (9) In a group of 100 men, 11 are tall and 89 are short. (10) In a group of 100 men, 11 are tall and 89 are short. (11) In a group of 100 men, 11 are tall and 89 are short. (12) In a group of 100 men, 11 are tall and 89 are short. (13) In a group of 100 men, 11 are tall and 89 are short. (14) In a group of 100 men, 11 are tall and 89 are short. (15) In a group of 100 men, 11 are tall and 89 are short. (16) In a group of 100 men, 11 are tall and 89 are short. 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The analysis of the distribution of hypertension furnishes a partial explanation of the decrease in incidence of tall men in the older ages as pointed out earlier in this paper. The greater hazard of hypertension among the tall broad men should cause an increase in their mortality in the earlier age groups. Costanzo¹⁰ says that "as the age at death increased, body structure became more and more short shaped." However, the vast number of short hypertensive persons should distort the mortality records to show a much higher death rate among short men. The Medico-Actuarial Mortality Investigation¹⁷ confirms this supposition in the statement that "at the younger ages tall men have proved less desirable risks than short men. At the older ages the short men . . . have been slightly worse risks than tall men." Dublin¹⁴ makes the same statement for women but adds in another article¹⁸: "Extremes of stature [both short and tall] are decided handicaps."

The predilection of hypertension for lateral build has also made its mark on the mortality, as recorded by chest measurements. As Dublin¹⁹ states, "... The lower mortality is shown in the chest-girth groups below the mean." He implies further that in any height group the smallest chest has the lower mortality. Since hypertensive heart disease plays such a large part in crude mortality, it would

seem that much of the differential mortality of height groups can be explained on the basis of the hazard of hypertension among lateral build persons and the greater proportion of lateral build in the persons of short stature.

CONCLUSIONS

1. A gross anthropologic study of 2,552 men and 2,021 women shows for the first time in medical literature the positive correlation of height to blood pressure.

2. Blood pressure is shown in this study to be affected by height. A review of the literature shows that no study, thus far, has found the height difference as reported in this paper. An explanation for this failure is probably due to a lack of correct statistical delineation of build types and the exclusive reliance upon mean and modal pressures, instead of a study of the incidence of low and high pressure distribution and the actual to expected ratio.

3. The incidence of tall men and women decreased steadily with an increase in age. This is an unusual and striking phenomenon, and is probably due chiefly to a high mortality of tall persons in earlier age groups.

4. In a random group of mixed builds and weights short men and women will show higher mean and modal systolic and diastolic blood pressures than tall men and women. Short men and women showed a higher incidence of high pressures than tall men and women.

5. When the build groups are separated and held constant, a marked difference in blood pressure is noted between tall and short persons and is reversed to the height relationship mentioned above.

6. The tall lateral or broad individual is more susceptible to hypertension than the short lateral one and is less likely to have a low pressure.

7. Tall lateral or broad men show an actual to expected ratio of about two and one-half times as many systolic and diastolic hypertensives as short lateral men.

8. Tall lateral women show twice as many systolic and diastolic hypertensives as short lateral women.

9. Tall lateral women show less than one-third the low systolic and diastolic pressures as short lateral women.

10. Tall lateral men have a slightly smaller incidence of low pressure than short lateral men.

11. The actual to expected ratio shows that among linear or thin men the tall men have only one-half as many low systolic and diastolic pressures as the short men.

12. The actual to expected ratio shows no difference between short and tall linear or thin women of low and high pressures.

13. In a previous paper it was shown that the lateral or broad build individual in any height group carried the highest incidence of hypertension. In this paper it is shown that lateral build is most often found among short men and women.

14. This unequal distribution of lateral build in short and tall persons explains the discrepancy mentioned in conclusion number 5. Although the tall person carries a greater hazard of hypertension than any other individual, the

larger number of lateral builds among short persons causes the bulk of the hypertensive population to be found among short persons.

15. The short individual shows a higher incidence of hypertension than the tall one in any weight group.

16. There are, therefore, three influencing height factors in hypertension. First, there is the difference in blood pressure between tall and short persons in specific build groups. This is purely a height difference. Second, there is the difference in pressure between tall and short persons when weight is held constant. Third, there is the difference in pressure between short and tall persons in any total group in which build and weight are naturally mixed. The second and third height differences are dependent upon the build factor.

REFERENCES

1. Alvarez, W. C., and Stanley, L. L.: Blood Pressure in Six Thousand Prisoners and Four Hundred Prison Guards. *A Statistical Analysis*, Arch. Int. Med. 46: 17, 1930.
2. Wunderlich, H.: Blood Pressure and Pulse Among Well Developed German Students and Their Connection With Various Body Measurements, *Ztschr. f. Konst.* 18: 349, 1934.
3. Reed, L. J., and Love, A. G.: Biometric Studies on United States Army Officers; Somatological Norms, Correlations and Changes With Age, *Human Biol.* 4: 509, 1932.
4. Stocks, P., and Karn, M. N.: Blood Pressure in Early Life, *Drapers' Company Research Memoirs*, London, 1924, Cambridge Univ. Press.
5. Hunter, A.: Blood Pressure, What Affects It? *Proceedings of the Seventeenth Annual Meeting of the Association of Life Insurance Presidents*, 1923.
6. Blood Pressure, Report of the Joint Committee on Mortality of the Association of Life Insurance Medical Directors and the Actuarial Society of America, New York, 1925.
7. Rucker, M. P., and Connell, J. W.: Blood Pressure in the New-Born, *Am. J. Dis. Child.* 27: 6, 1924.
8. Ritchey, H. G.: Blood Pressure in Boys and Girls Before and After Puberty and Its Relation to Growth and Maturity, *Am. J. Dis. Child.* 42: 1281, 1931.
9. Fishberg, A. M.: Hypertension and Nephritis, ed. 2, Philadelphia, 1931, Lea & Febiger.
10. Robinson, S. C., and Brucer, M.: The Range of Normal Blood Pressure, *Arch. Int. Med.* 64: 409, 1939.
11. Robinson, S. C., Brucer, M., and Mass, J.: Hypertension and Obesity, *J. LAB. & CLIN. MED.* 25: 807, 1940.
12. Robinson, S. C., and Brucer, M.: Body Build and Hypertension, *Arch. Int. Med.* 66: 393, 1940.
13. Medico-Actuarial Mortality Investigation, Vol. I, *The Association of Life Insurance Medical Directors and the Actuarial Society of America*, New York, 1912.
14. Dublin, L. I., and Marks, H. H.: The Build of Women and Its Relation to Their Mortality (Read at the Forty-eighth Annual Meeting of the Association of Life Insurance Medical Directors of America, October 28-29, 1937), New York, 1938, Press of Recording and Statistical Corporation.
15. Draper, G.: *Disease and the Man*, New York, 1930, The Macmillan Co.
16. Costanzo, A.: *Constitution and Mortality*, Milan, 1936, Catholic University of the Sacred Heart.
17. Medico-Actuarial Mortality Investigation, Vol. II, *The Association of Life Insurance Medical Directors and the Actuarial Society of America*, New York, 1913.
18. Dublin, L. I.: Body Build and Longevity, *The De Lamar Lectures*, 1925-1926.
19. Dublin, L. I.: Third Report of the Committee on Dreyer Measurements in Relation to Life Insurance Underwriting Practice. III. Anthropomorphic and Mortality Tables for Overweights. Chest Girth in Relation to Relative Spine Length, New York, 1923, Metropolitan Life Press.

STUDIES ON EXPERIMENTAL LESIONS IN THE KIDNEYS OF RABBITS*

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THE purpose of this paper is to report the results of investigations on the production of experimental renal lesions in rabbits by homologous nephrotoxins and certain bacterial filtrates. The work of Shwartzman,^{1, 2} Gerber,³ and Apitz⁴ indicates that it is possible to damage rabbit kidneys by two or more injections of selected bacterial filtrates into the systemic circulation. Masugi⁵ showed that experimental lesions could be produced in the kidneys of rabbits by injection of duck anti-rabbit kidney serum. Smadel⁶⁻⁹ has reported similar results in rats by the use of rabbit anti-rat kidney serum. These lesions bore a striking similarity to those of human glomerulonephritis. To get a potent anti-kidney serum it was necessary to use two species of animals. Schwentker and Rivers¹⁰ showed that repeated injections of autolyzed rabbit brain into rabbits produced complement-fixing antibodies. This finding suggested that by autolyzing homologous protein, one could render it antigenic to the same species of animal. An attempt was made, therefore, to autolyze renal protein by tying off the renal artery of a rabbit's kidney and allowing it to degenerate in vivo. In the experiments to be reported rabbits were injected with emulsions of such degenerated kidney and their serum was used in an attempt to produce renal changes in other rabbits.

Other experiments were conducted in an attempt to damage renal proteins by a Shwartzman reaction.

EXPERIMENTS ON DEGENERATED RABBIT KIDNEY

Under ether anesthesia the left renal artery of a rabbit was ligated. Eighteen hours after ligation the rabbit was killed and both kidneys were removed aseptically. The left kidney was definitely swollen and discolored. The normal right kidney was perfused through the renal artery with sterile normal saline in order to remove the blood remaining in it at the time of death. No attempt was made to perfuse the degenerated kidney. Each kidney was then cut up aseptically with scissors and macerated in a mortar. Sterile emulsions of each were made by shaking up the macerated kidneys with 50 c.c. of sterile saline. Two rabbits then received seven intraperitoneal injections each of the two emulsions at daily intervals and were killed by bleeding to death. The blood from each rabbit was preserved carefully. On autopsy neither of these injected animals showed significant pathologic changes in the kidneys or other organs.

The precipitin titer of the blood of these two injected rabbits was examined by means of Wright's capillary-tube technique. It was found that precipitating

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antibodies to the dilutions of the ischemic kidney emulsion were present in significant amounts in the blood of the rabbit that had received that emulsion. The blood of the control rabbit that had received injections of normal kidney emulsion contained no precipitating antibodies.

Two other rabbits were subjected to intravenous injections of the precipitating rabbit serum. One received a single intravenous dose of 3 c.c. of serum and was killed nineteen hours later. Autopsy revealed no pathologic change in the kidneys or other organs. A second rabbit received six intravenous injections of the precipitating serum daily and was killed thirty-two hours after the last injection. On autopsy the kidneys showed no significant lesions. It was concluded from these experiments that, although the serum injected contained precipitating antibodies, there was no evidence of its being nephrotoxic in the sense of producing nephritis.

EXPERIMENTS WITH BACTERIAL FILTRATES

The bacterial filtrates used in these experiments were prepared from cultures of typhoid bacilli and hemolytic streptococci. Typhoid organisms were from a stock culture and the filtrates were prepared by the method of Shwartzman. These bacteria were grown on large surfaces of agar and washed off with normal salt solution. The washings were centrifuged and the supernatant fluid was passed through a Berkefeld filter. The resulting filtrate was used for the animal injections. In earlier experiments the streptococci used were obtained from an infected human knee joint. Those used in the last group of experiments were a group A hemolytic strain obtained through the kindness of Dr. Ronald Hare, of the Connaught Laboratories, University of Toronto. In both cases they were grown in broth and filtered through Berkefeld candles.

The technique of injection of the left renal artery was as follows: Under ether anesthesia a longitudinal lumbar incision was made. The lumbar abdominal muscles were split in gridiron fashion, and the artery and vein were exposed without perforating the peritoneum. Both vein and artery were freed of connective tissue, and a ligature was passed around the vein but not tied. The artery was elevated on a hook at the same time the vein was temporarily occluded by traction on the venous ligature. The artery was injected then by using a syringe and a bent needle. The volume of fluid injected was usually 2 c.c. A successful injection of the artery was evidenced by a distinct pallor of the kidney which could be seen through the peritoneum. The needle then was withdrawn and the hook occluding the artery was removed. Hemorrhage from the puncture in the arterial wall was controlled by a sponge soaked in saline at a temperature of about 44° C. The vein was kept occluded for five minutes in order to prevent the injected fluid from being washed out of the kidney immediately. The venous ligature was removed then and the circulation within the kidney allowed to re-establish itself. The kidney, which was blue and tense at the moment of release, would resume quickly its normal chocolate brown color and become much softer to the touch. The muscles of the loin were repaired and the wound was closed. Rabbits so treated showed no systemic ill effects.

EXPERIMENTAL RESULTS

(1) *Intra-Arterial Streptococcal Filtrate Alone.*—One rabbit received an injection of 1.5 c.c. of the first streptococcal filtrate in its left renal artery. No further injections were made, and the rabbit was killed twenty-nine hours later. Macroscopically the left kidney was slightly paler than the right, but microscopically no significant changes could be found.

(2) *Intra-Arterial Streptococcal Filtrate Followed by Single Intravenous Streptococcal Filtrate.*—The left renal artery of a rabbit was injected with 1.5 c.c. of the same streptococcal filtrate. In twenty-four hours it was given an intravenous injection of 1.5 c.c. of this streptococcal filtrate in the ear vein; the rabbit was killed four hours later. On autopsy the left kidney was paler than the right. Microscopically neither kidney showed any definite abnormality.

(3) *Intra-Arterial Typhoid Filtrate Followed by Single Intravenous Streptococcal Filtrate.*—The left renal artery of a rabbit was injected with 1.5 c.c. of the first typhoid filtrate. Twenty-four hours later an injection of 3 c.c. of the first streptococcal filtrate was made in the ear vein. The animal was killed five hours later. On autopsy the left kidney was pale and swollen while the right kidney was normal. The left kidney weighed 15.5 Gm., and the right kidney 11.5 Gm. Microscopically the left kidney showed little pronounced change in the glomeruli. Surrounding the capsule of a number of glomeruli was a zone of hyaline material which contained no nuclei; it stained pink with hematoxylin and eosin and red with Heidenhain's azocarmine aniline blue. There was disintegration of many proximal tubules with disappearance of nuclei, and similar damage was observed in some of the distal convoluted tubules. Many tubules down to the pelvis were seen to contain hyaline material similar to that described as surrounding some of the glomerular capsules. In this kidney there was damage to the tubules with exudation within as well as outside the nephron. No significant microscopic changes were observed in the right kidney. The liver showed fatty degeneration.

A second rabbit of this group received an injection of 2 c.c. of the second typhoid filtrate in its left renal artery. Twenty-four hours later it was given an intravenous injection of 2 c.c. of the first streptococcal filtrate in the ear vein. It died in about twenty-six hours. In the gross and microscopically both kidneys were normal.

(4) *Intra-Arterial Typhoid Filtrate Followed by Single Intravenous Typhoid Filtrate.*—A rabbit received 2 c.c. of the first typhoid filtrate in its left renal artery and 0.25 c.c. in the skin of the abdomen. At the end of twenty-four hours 3 c.c. of this typhoid filtrate were injected into the ear vein. The animal was killed five hours later. On autopsy the left kidney was slightly pale, and the skin showed no significant erythema. Microscopically some patchy areas of ischemic atrophy were seen in the left kidney. The right kidney was normal. No change was observed in either kidney that might not have been quite spontaneous in the rabbit.

A second rabbit of this group likewise was injected in its left renal artery with 2.5 c.c. of the second typhoid filtrate. At the same time an injection of 0.5 c.c. was made in the skin. Twenty-four hours later, at the time when the skin

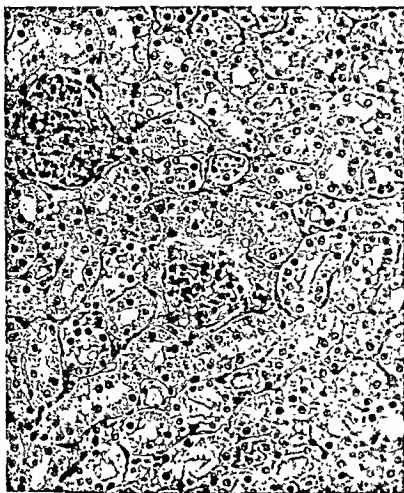


Fig. 1—First rabbit of Experiment 3. Left kidney showing exudate about glomeruli and in some tubules.



Fig. 2—First rabbit of Experiment 3. Left kidney showing slightly damaged proximal tubules.

showed a slight erythema, an injection of 2 c.c. of the same second typhoid filtrate was made in the ear vein. The rabbit died three hours later. On autopsy the kidneys showed nothing of note.

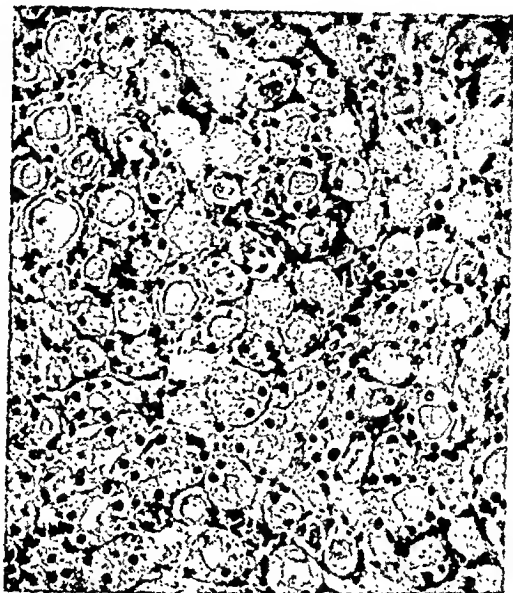


Fig. 3.—First rabbit of Experiment 3. Left kidney showing loop tubules containing exudate.

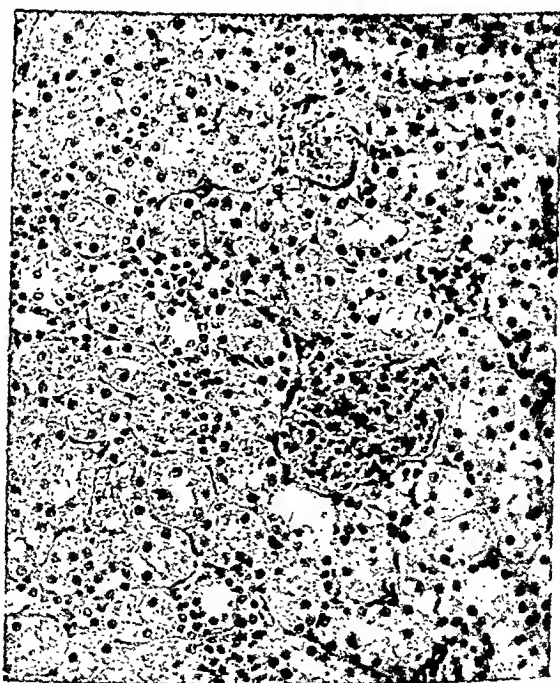


Fig. 4.—First rabbit of Experiment 3. Untouched right kidney—undamaged.

(5) *Intra-Arterial Typhoid Filtrate Followed by Multiple Intravenous Streptococcal Filtrate Injections.*—A single rabbit was given 2 c.c. of a third typhoid filtrate in the left renal artery. Twenty-four hours later it received

an intravenous injection of 2 c.c. of the second streptococcal filtrate. These intravenous streptococcal filtrate injections were repeated once daily until 27 in all had been given. The rabbit was then killed. On autopsy the left kidney appeared pale and granular, and on section had a rather narrow cortex. The right kidney was normal. On microscopic section the left kidney showed marked focal fibrous atrophy. There were no glomerular lesions of note. The right kidney was normal microscopically. The lesions in the left kidney may have been due to the production of thromboses at the time of the arterial injection.

(6) *Intra-Arterial Typhoid Filtrate Followed by Multiple Intravenous Typhoid Filtrate Injections.*—A rabbit was given an injection of 2 c.c. of the third typhoid filtrate in its left renal artery. Twenty-four hours later it received 2 c.c. of the same filtrate in the ear vein, and thereafter daily intravenous injections of 2 c.c. of the same filtrate until 16 in all had been given. The rabbit was killed. On autopsy the left kidney was pale and had a large infarct. The right kidney was normal. Microscopically, apart from the infarct, there was much focal fibrous atrophy of the left kidney. The right kidney was normal.

(7) *Intra-Arterial Typhoid Followed by Multiple Intravenous Streptococcal Injections, Using Controls and Varying Time Intervals.*—Nine rabbits were used in the experiment subdivided into three groups of three. The first two groups were controls and the third the experimental group. The rabbits of the first group each received a single injection of 2 c.c. of the fourth typhoid filtrate in their left renal arteries. The rabbits of the second group each received daily intravenous injections of 2 c.c. of streptococcal filtrate but no antecedent arterial injections. The rabbits of the third, or experimental, group each received an injection of 2 c.c. of the typhoid filtrate in the left renal artery followed by daily intravenous injections of 2 c.c. of streptococcal filtrate. The first rabbit of each group was killed ten days after the initial injection, the second twenty days, and the third thirty days after that injection. The results were disappointing. No significant changes, gross or microscopic, were produced in the kidneys of any of the animals. Minor changes, such as focal fibrous atrophy, edema, and interstitial eosinophilic infiltration, were observed, but nothing that does not occur spontaneously in rabbits. It would appear that the treatment accorded these rabbits, control or experimental, was without effect in producing glomerular changes. Certainly nothing of the nature of glomerulonephritis was observed.

SUMMARY AND CONCLUSIONS

An attempt was made to produce pathologic changes in the kidneys of rabbits by intravenous injections of serum from rabbits that had been treated with intraperitoneal injections of degenerated rabbit kidney emulsion. An attempt was also made to damage rabbit kidney by bacterial filtrates. The results of these investigations were essentially negative. One interesting observation, from a serologic point of view, was the finding of precipitating antibodies in the serum of a rabbit that had been injected intraperitoneally with emulsions of degenerated rabbit kidney. Such precipitating antibodies could not be demonstrated in the blood of a rabbit that had received injections of a normal kidney emulsion. The renal lesions encountered in one rabbit described, while interest-

ing, could not be reproduced. The interpretation of the changes in this single rabbit must remain an open question. The possibility of an allergic reaction may be considered, but it could not be denied that a circulatory disturbance in handling the kidney might have been responsible.

REFERENCES

1. Shwartzman, G.: Phenomenon of Local Tissue Reactivity, New York, 1937, Paul B. Hoeber, Inc., p. 193.
2. Shwartzman, G.: The Phenomenon of Local Skin Reactivity to Bacterial Filtrates: Elicitation of Local Reactivity by Way of the Vascular System, *J. Exper. Med.* 62: 621, 1935.
3. Gerber, I. E.: Morphological Aspects of the Local Shwartzman Phenomenon, *Arch. Path.* 21: 331, 1936; The Shwartzman Phenomenon in the Kidneys of Rabbits—Observations on Effects of Intravenous Administration of Bacterial Filtrates, *Arch. Path.* 21: 776, 1936.
4. Apitz, K.: Die Wirkung bakterieller Kulturfiltrate nach Umstimmung des gesamten Endothels beim Kaninchen, *Virchows Arch. f. path. Anat.* 293: 1, 1934.
5. Masugi, M.: Ueber die experimentelle Glomerulonephritis durch das spezifische Antinierenserum, *Beitr. z. path. Anat. u. z. Allg. Path.* 92: 429, 1934.
6. Smadel, J. E.: Experimental Nephritis in Rats Induced by Injection of Anti-Kidney Serum. I. Preparation and Immunological Studies of Nephrotoxin, *J. Exper. Med.* 64: 921, 1936.
7. Smadel, J. E., and Farr, L. E.: Experimental Nephritis in Rats Induced by Injection of Anti-Kidney Serum. II. Clinical and Functional Studies, *J. Exper. Med.* 65: 527, 1937.
8. Smadel, J. E.: Experimental Nephritis in Rats Induced by Injection of Anti-Kidney Serum. III. Pathological Studies of the Acute and Chronic Disease, *J. Exper. Med.* 65: 541, 1937.
9. Swift, H. F., and Smadel, J. E.: Experimental Nephritis in Rats Induced by Injection of Anti-Kidney Serum. IV. Prevention of the Injurious Effects of Nephrotoxin in Vivo by Kidney Extract, *J. Exper. Med.* 65: 557, 1937.
10. Schwentker, F. F., and Rivers, T. M.: The Antibody Response of Rabbits to Injections of Emulsions and Extracts of Homologous Brain, *J. Exper. Med.* 60: 559, 1934.
11. Horn, Henry: The Experimental Nephropathies, General Reviews, *Arch. Path.* 23: 71, 1937; *Arch. Path.* 23: 241, 1937.
12. McCann, W. S.: Bright's Disease, A Review of Recent Literature, *Arch. Int. Med.* 60: 167, 1937.
13. McCann, W. S.: Bright's Disease, A Review of Recent Literature, *Arch. Int. Med.* 61: 501, 1938.

DISTURBANCES IN THE BLOOD FOLLOWING EXPOSURE TO BENZOL*

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INTRODUCTION

THE fact that benzol when taken into the human body may have deleterious effects upon the hematopoietic system has been known for about forty years.¹ With the exception of lead, there is probably no industrial poison better known to physicians than benzol. A rather extensive literature on the subject of benzol poisoning has accumulated, and most of the reports, especially the more recent ones, contain some mention of the blood picture. The most extensive review of the subject yet to appear is that of the British Industrial Health Research Board.² Another comprehensive review is that of Hamilton,³ published in 1931. The present status of knowledge concerning the blood picture in chronic benzol poisoning is well summed up in the following lines taken from the British report just mentioned: "While the most characteristic blood picture in benzol poisoning remains that described by the earlier authors, a leucopenia with neutropenia, thrombopenia and some anemia, it becomes increasingly evident that many cases present wide variations on this picture."

Most cases of benzol poisoning described in the literature have been fatal or at least of such severe degree that hospitalization has been necessary. Cases of this type are frequently complicated by hemorrhage, infection, or some other secondary factor. Whereas acute poisoning has been well differentiated from the chronic form, little distinction has been made between mild and severe forms of chronic poisoning. Relatively few observers have had an opportunity to study more than two or three isolated cases of chronic benzol poisoning, and not many of these have included a complete set of hematologic observations in their studies. Incomplete observations by a large number of physicians using different methods and terminologies and describing small numbers of cases must result in some confusion.

In the literature dealing with industrial poisoning, control studies are practically never mentioned. Benzol poisoning is no exception to this general rule. A careful observer would never consider publishing results of experimental poisoning in laboratory animals without having performed control experiments, even though "normal" values for the species of animal were supposed to be well known. In industrial poisoning the experimental animal is a human being. It seems entirely illogical to neglect establishing normals for the apparatus and methods in the hands of the particular individual who is making the study.

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This paper presents the results of comprehensive hematologic studies on a large group of individuals who had been exposed to benzol. The significance of the results is enhanced by the inclusion of controls.

CASES STUDIED

The control group used in this investigation consisted of 81 male industrial workers employed in a factory where no occupational hazard could be demonstrated. These workers varied in age between 20 and 65 years. None gave a history of abnormal symptoms and none had any significant abnormal findings on physical examination. For all practical purposes the group could reasonably be considered to be a small but representative sample of the industrial population employed in a nonhazardous industry in New York City. A control series in the industry in which the benzol poisoning occurred was not possible, since no branch of that particular work is free of toxic materials.

TABLE I

TESTS PERFORMED AND NUMBER OF INDIVIDUALS SUBJECTED TO EACH TEST

BLOOD DETERMINATION	CONTROL	BENZOL	TOTAL
Hemoglobin	81	235	316
Erythrocytes	81	159*	240
Leucocytes	81	332*	413
Differential	81	112	193
Platelets	50	107	157
Reticulocytes	47	103	150
Bleeding time	52	105	157
Coagulation time	52	99	151
Sedimentation rate	59	104	163
Hematocrit	81	104	185
Mean corpuscle volume	75	103	178
Mean corpuscle hemoglobin	73	120	193
Mean corpuscle hemoglobin concentration	81	105	186
Fragility of red blood cells	68	92	160
Serum bilirubin	0	102	102

*Thirty-seven erythrocyte counts and 98 leucocyte counts were made by Dr. B. Mintz of the Division of Women in Industry, of the New York State Department of Labor. I made all other determinations.

The workers exposed to benzol (henceforth referred to as the benzol group) were employed in the rotogravure printing industry in New York City. A full description of this industry and its hazards has been given elsewhere. Suffice it to say here that every individual in the group had been exposed to concentrations of benzol fumes ranging from 11 to 1,060 p.p.m. for a period of at least six months, and in most instances for about three years.* In all, 33 rotogravure workers were examined. A complete set of hematologic observation was made in about 100 cases. The tests performed and the number of individuals subjected to each test are given in Table I. The age distribution of the control and benzol groups is shown in Table II. The differences between the two groups are not so great that they render comparison fallacious.

*The inks used in the particular type of rotogravure printing with which we are concerned contained chemicals other than benzol which might conceivably have produced toxic effects. Since benzol was the predominant solvent and since the effects were compatible with benzol intoxication, the other ingredients are not considered to be of great etiologic significance. The other solvents, all of which were used in relatively small amounts, were toluene, xylene, methyl ethyl ketone, petroleum naphtha, ethyl, butyl, and amyl acetate, and butyl and amyl alcohol.

Since there was no direct relationship between the duration of employment and the nature of the blood findings (Table III), this feature will not be discussed. All the individuals in the benzol group were actually working at the time they were examined. As a result of the blood studies, six were considered poisoned seriously enough to warrant hospitalization. Further studies on these patients have recently been reported.⁶ About 100 were referred to various physicians for observation and treatment. All but the six hospitalized persons were allowed to continue to work inasmuch as the use of benzol was discontinued shortly after this study was begun.

TABLE II
AGE DISTRIBUTION*

AGE GROUP	CONTROL		BENZOL	
	NUMBER	PER CENT	NUMBER	PER CENT
20-29 years	33	41.2	154	49.4
30-39 years	12	15.0	86	27.6
40-49 years	21	26.3	52	16.6
50-59 years	8	10.0	15	4.8
60-69 years	6	7.5	5	1.6
Total	80	100.0	312	100.0

*In a few cases the age was not ascertained.

TABLE III

CORRELATION OF LENGTH OF EXPOSURE AND BENZOL EFFECT

The differences in the various exposure groups are not statistically significant (chi square test)

BLOOD PICTURE	TIME OF EXPOSURE IN MONTHS							
	0-12 MO.		13-24 MO.		25-36 MO.		37-60 MO.	
	NUMBER	PER CENT	NUMBER	PER CENT	NUMBER	PER CENT	NUMBER	PER CENT
Normal	24	63.2	22	46.8	97	55.8	34	60.7
Abnormal	14	36.8	25	53.2	77	44.2	22	39.3
Total	38	100.0	47	100.0	174	100.0	56	100.0

METHODS

Erythrocyte and leucocyte counts were performed on capillary blood drawn from the finger tip. Bureau of Standards certified pipettes were used. For hemoglobin determinations the Newcomer method was used and readings were made on a photoelectric colorimeter.* This instrument was considered to offer many advantages, since on certain days as many as 30 or 40 hemoglobin determinations had to be made. Blood smears stained with Wright's stain were used for the differential counts, and a slight modification of Schilling's⁶ classification followed. In previous studies on smaller groups of subjects 200 cells were counted. Close agreement was found between the distribution of cells in the first and second hundreds so that in the present study, involving so many subjects, this refinement of technique was not observed.

Reticulocytes were vitally stained on slides previously prepared with a saturated alcoholic solution of brilliant cresyl blue. The number of reticulated cells observed while counting 1,000 erythrocytes was used as the basis for calculating the percentage of reticulocytes. Blood platelets were estimated from stained

*Kindly loaned by the Klett Colorimeter Company, New York.

smears of capillary blood drawn through a drop of 14 per cent magnesium sulfate solution, the smears being stained with Wright's stain. The number of thrombocytes observed while counting 1,000 erythrocytes was used as the basis for calculation.

The bleeding time was determined by Duke's method.⁷ Blood was drawn from the lobe of the ear by means of an automatic lancet adjusted to produce a uniform depth of incision. Coagulation time was measured on venous blood by the method of Lee and White.⁷ For calculating the sedimentation rate of the erythrocytes the method of Wintrobe and Landsberg⁸ was followed and the results were corrected for anemia. A mixture of ammonium and potassium oxalate was used as the anticoagulant. The hematocrit value was obtained in the same tube that was used for the sedimentation rate, and this value was used in calculating the mean corpuscular volume and the mean corpuscular hemoglobin concentration. These indices, as well as the mean corpuscular hemoglobin content, were obtained according to the methods of Wintrobe.⁹ To produce maximum packing of the cells, the hematocrit tubes were centrifuged at 3,600 r.p.m. for one hour.¹⁰ Fragility of the erythrocytes (resistance to hypotonic saline) was tested by the method of Sanford⁷ and serum bilirubin was determined as described by Ernst and Förster.¹⁰

Checking of Results.—In the controls as well as in the benzol group, where results of any test showed decided deviation from the normal, the test was repeated in many instances on a fresh specimen of blood. In a few cases repetition was not possible. Almost invariably the second test agreed closely with the first. Where there was disagreement, the figure nearer to normal was accepted.

NORMAL STANDARDS

In the present study it was not necessary to rely on the figures given in the literature for normal blood values, since a reasonably large control group comprised a part of this investigation. Most of the "normal" groups that have been reported have been made up of students, nurses, and similar types of individuals. Little has been written concerning the "normal" blood picture of industrial workers. There is no reason to believe that standards for workers in nonhazardous industries differ greatly from those of student groups. To support this view, Table IV has been prepared using Osgood's¹¹ figures for the purpose of comparison. The only significant difference seems to be in the hemoglobin values, there being no obvious reason for the discrepancy other than difference in method. Particular attention is called to the values for lymphocyte percentages. Most writers who have described blood findings in connection with industrial poisoning seem to have accepted the old idea that lymphocytes normally comprise 20 to 30 per cent of the leucocytes. As a result, practically every industrial poison is said to be associated with a relative lymphocytosis. If it is held that any value above 30 per cent of lymphocytes constitutes a lymphocytosis, one is quite certain to find a "lymphocytosis" in a large proportion

*In previous studies it had been observed that centrifugation at 3,600 r.p.m. for thirty minutes, as recommended by Wintrobe, did not always produce constant volume. It is possible that the modification used in the present study was responsible for the fact that the mean corpuscular volume range in the control series was lower than that reported by Wintrobe as normal.

globin were found only in those cases which had evidence of severe injury to the hematopoietic system. The results indicate that a low hemoglobin value is not a prominent part of the picture of chronic benzol exposure and in general is present only when the erythrocytes have fallen to a very low level. As will be pointed out later the hemoglobin values remained relatively high in relation to the erythrocytes. In other words, hyperchromia of the red corpuscles was the rule.

TABLE VI

DISTRIBUTION OF ERYTHROCYTE COUNTS IN CONTROL AND BENZOL GROUPS

ERYTHROCYTES (MILLIONS PER C.M.M.)	CONTROL		BENZOL	
	NUMBER	PER CENT	NUMBER	PER CENT
1.5-1.99	0	0	1	0.6
2.0-2.49	0	0	1	0.6
2.5-2.99	0	0	3	1.9
3.0-3.49	0	0	3	1.9
3.5-3.99	0	0	22	13.9
4.0-4.49	2*	2.4	46	29.0
4.5-4.99	16	19.8	50	31.4
5.0-5.49	33	40.8	29	18.2
5.5-5.99	28	34.6	4	2.5
6.0-6.49	2	2.4	0	0
Total	81	100.0	159	100.0

*These two counts were 4.47 and 4.48 millions, respectively.

Erythrocytes.—Details of the erythrocyte counts are given in Table VI. Whereas but two individuals in the control group fell below 4.5 million (and these two but very slightly below), 76 workers, or 47.9 per cent, of those exposed to benzol whose red blood cells were counted had subnormal erythrocyte values. It is obvious, therefore, that a diminution in red corpuscles is a relatively frequent finding in chronic benzol exposure. The polycythemia described by certain observers,^{13, 14} in early benzol poisoning was not found in the present series. It is possible that if erythrocyte counts had been done on the entire group, some higher counts might have been encountered. On the other hand, since the individuals studied had been exposed from six to sixty months to concentration of 11 to 1,060 p.p.m., they may reasonably be said to represent all degrees of benzol effect, ranging from none at all to severe damage. It would seem that polycythemia, if it occurs in benzol exposure, should have appeared in at least a few members of the benzol group.

Qualitative changes in the erythrocytes, such as poikilocytosis, basophilic stippling and nucleated forms, were seen only in cases in which the degree of anemia was severe. All the severely anemic cases, however, did not exhibit these qualitative abnormalities. No effort was made to estimate the size of the red corpuscles from stained blood films, since it was felt that simple inspection was not entirely reliable and use of the Price-Jones technique was too time-consuming for a series as large as the present one. The average size of the erythrocyte (mean corpuscular volume) was calculated according to the method of Wintrobe. As will be shown later there was a definite tendency to macrocytosis in the benzol group.

Leucocytes.—As was stated in the introductory remarks, a leucopenia has always been considered one of the most characteristic features of benzol poison-

ing. If one considers only cases of an extreme degree of poisoning, this view is undoubtedly correct. If, however, reliance is placed on the finding of leucopenia to diagnose mild or early cases, serious error might result. In this connection Hamilton¹⁵ has stated that "if the physician depends on a leucopenia for the diagnosis and fails to make a count of the red cells, the patient may be fatally poisoned before he discovers that benzol poisoning has caused marrow aplasia." It can be seen in Table VII that only 14.5 per cent of the benzol group had less than 5,000 white blood cells and 69 per cent had more than 6,000 leucocytes. It might be argued that those patients having normal white cell counts were not really poisoned, but to sustain such a point of view one would have to contend that anemia, macrocytosis, and thrombocytopenia were no evidence of benzol poisoning.

TABLE VII

DISTRIBUTION OF LEUCOCYTE COUNTS IN CONTROL AND BENZOL GROUPS

LEUCOCYTES (PER C.M.M.)	CONTROL		BENZOL	
	NUMBER	PER CENT	NUMBER	PER CENT
2,000-2,999	0	0	6	1.8
3,000-3,999	0	0	16	4.8
4,000-4,999	0	0	26	7.9
5,000-5,999	7	8.6	55	16.5
6,000-6,999	19	23.4	58	17.5
7,000-7,999	16	19.8	55	16.5
8,000-8,999	11	13.6	48	14.6
9,000-9,999	10	12.4	24	7.2
10,000-10,999	13	16.1	26	7.9
11,000-11,999	3	3.7	7	2.1
12,000-12,999	1	1.2	4	1.2
13,000-13,999	0	0	3	0.9
14,000-14,999	1	1.2	2	0.6
15,000-15,999	0	0	0	0
16,000-16,999	0	0	2	0.6
Total	81	100.0	332	100.0

It has occasionally been stated that leucocytosis is found in early benzol poisoning. The incidence of high white blood cell counts in the present series was not sufficiently frequent to support the view that leucocytosis is a characteristic feature in benzol exposure.

Lymphocytes.—Mention has already been made of the frequency with which relative lymphocytosis has been described in connection with industrial poisoning in general and benzol poisoning in particular. It is felt that the commonly accepted normal range of lymphocytes is far too low and that this misconception of what is normal has resulted in a certain amount of confusion. Inspection of Table VIII shows that lymphocyte percentages above 40 occurred in more than one-third of the control group, and that values above 50 per cent of lymphocytes were by no means rare. Further, only 12.4 per cent of the control group had between 20 and 30 per cent of lymphocytes. It has been pointed out in Table IV that the figures for range and average of this control group were very close to those given by Osgood^{11, 12} as his normals.

On comparing the benzol group with the control group (Table VIII) perhaps the most striking feature is that the incidence of lymphocyte values between 20 and 30 per cent, the traditional normal, was nearly three times as great in

TABLE VIII

DISTRIBUTION OF LYMPHOCYTE PERCENTAGES IN CONTROL AND BENZOL GROUPS

LYMPHOCYTES (PER CENT)	CONTROL		BENZOL	
	NUMBER	PER CENT	NUMBER	PER CENT
10-19	2	2.4	10	8.9
20-29	10	12.4	41	36.6
30-39	40	49.5	41	36.6
40-49	23	28.3	15	13.4
50-59	5	6.2	5	4.5
60-69	1	1.2	0	0
Total	81	100.0	112	100.0

TABLE IX

DISTRIBUTION OF ABSOLUTE LYMPHOCYTE VALUES IN CONTROL AND BENZOL GROUPS

LYMPHOCYTES (ABSOLUTE NUMBERS)	CONTROL		BENZOL	
	NUMBER	PER CENT	NUMBER	PER CENT
750-1,000	0	0	7	6.2
1,000-1,999	9	11.1	54	48.3
2,000-2,999	34	42.0	39	34.8
3,000-3,999	20	24.8	8	7.1
4,000-4,999	14	17.2	4	3.6
5,000-5,999	3	3.7	0	0
6,000-6,100	1	1.2	0	0
Total	81	100.0	112	100.0

TABLE X

DISTRIBUTION OF MONOCYTE AND EOSINOPHILE PERCENTAGES IN CONTROL AND BENZOL GROUPS

A				
MONOCYTES (PER CENT)	CONTROL		BENZOL	
	NUMBER	PER CENT	NUMBER	PER CENT
0-5	39	48.2	55	49.1
6-10	39	48.2	49	43.7
More than 10	3	3.6	8	7.2
Total	81	100.0	112	100.0

B				
EOSINOPHILES (PER CENT)	CONTROL		BENZOL	
	NUMBER	PER CENT	NUMBER	PER CENT
0-3	64	79.0	82	73.2
4-7	15	18.5	30	26.8
More than 7	2	2.5	0	0
Total	81	100.0	112	100.0

TABLE XI

DISTRIBUTION OF PLATELET COUNTS IN CONTROL AND BENZOL GROUPS

PLATELETS (PER C.MM.)	CONTROL		BENZOL	
	NUMBER	PER CENT	NUMBER	PER CENT
14,000- 50,000	0	0	12	11.2
50,000- 99,000	0	0	23	21.5
100,000-149,000	9	18.0	31	28.9
150,000-199,000	11	22.0	20	18.7
200,000-249,000	20	40.0	12	11.2
250,000-299,000	8	16.0	6	5.6
300,000-380,000	2	4.0	3	2.8
Total	50	100.0	107	100.0

the benzol group as in the control group, whereas values above 40 per cent were twice as frequent in the controls. These findings can lead to but one conclusion, namely, that exposure to benzol produces, if anything, a relative *lymphopenia*. That this lymphopenia is absolute as well as relative is shown in Table IX. It will be pointed out later that in the present study relative lymphocytosis was not found even in those cases which showed evidence of severe damage to the hematopoietic system. It would seem that a frank relative lymphocytosis occurs with some frequency only in those cases of benzol poisoning which terminate fatally with a fully developed picture of aplastic anemia.

Differential Formula.—Except for the lymphocytes, no features in the differential formula require any extended comment. The neutropenia, so prominently featured in the literature, is based on the same questionable standards as the alleged lymphocytosis and so requires no further discussion. In performing the differential counts by Schilling's⁶ method, it was apparent that there was no significant shift of the polymorphonuclear cells either to the left or to the right. For this reason, there seemed to be no indication for doing Cooke or Arnetz counts.

As can be seen in Table X monocytosis and eosinophilia did not occur in the benzol group nor were unusual numbers of basophiles found. Myeloblasts or myelocytes were not seen in any of the blood smears that were examined.

Blood Platelets.—A reduction in the number of blood platelets was a frequent and significant finding in the group exposed to benzol. Table XI shows that 32.7 per cent of the benzol group had less than 100,000 platelets, while only 19.6 per cent had counts above 200,000. In the control group there were no counts under 100,000, and 60 per cent were above 200,000. The difference between the two groups was marked enough to indicate that a diminution in thrombocytes is an important part of the blood picture resulting from benzol exposure.

TABLE XII

DISTRIBUTION OF RETICULOCYTE PERCENTAGES IN CONTROL AND BENZOL GROUPS

RETICULOCYTES (PER CENT)	CONTROL		BENZOL	
	NUMBER	PER CENT	NUMBER	PER CENT
0-0.5	16	34.1	40	38.8
0.6-1.0	28	59.5	38	36.9
1.1-2.0	3	6.4	22	21.4
More than 2.0	0	0	3*	2.9
Total	47	100.0	103	100.0

*The highest value was 2.4 per cent.

Reticulocytes.—As can be seen in Table XII the reticulocyte counts tended to be slightly higher in the benzol group than in the control group. In general, the higher values were found in those individuals who showed evidence of the most severe degree of damage to the hematopoietic system. No really significant increase in reticulocytes was encountered in the entire series.

Bleeding Time.—Table XIII shows some tendency toward prolongation of the bleeding time in the benzol group, but outspoken abnormality was rare. The two individuals whose bleeding time was longer than fifteen minutes had 46,000

and 30,000 blood platelets, respectively. These were by no means the lowest platelet counts found in the series. Of particular interest is the fact that although in 12 cases thrombocytes numbered less than 50,000, and in 35 cases were below 100,000, only two individuals had excessively long bleeding times, and in only three more was the bleeding time moderately prolonged. It is apparent that following benzol exposure it is possible for the platelets to reach a very low level without any change in the bleeding time.

TABLE XIII

DISTRIBUTION OF BLEEDING TIMES IN CONTROL AND BENZOL GROUPS

BLEEDING TIME (MINUTES)	CONTROL		BENZOL	
	NUMBER	PER CENT	NUMBER	PER CENT
Less than 2	43	82.7	55	52.4
2-4	9	7.3	45	42.8
4.5-6	0	0	3	2.9
More than 6	0	0	2*	1.9
Total	52	100.0	105	100.0

*In these two cases the bleeding was controlled by local pressure after fifteen minutes

TABLE XIV

DISTRIBUTION OF COAGULATION TIMES IN CONTROL AND BENZOL GROUPS

COAGULATION TIME (MINUTES)	CONTROL		BENZOL	
	NUMBER	PER CENT	NUMBER	PER CENT
Less than 9	10	19.2	29	29.3
9-12	34	65.5	53	53.5
13-16	7	13.4	13	13.1
More than 16	1	1.9	4	4.1
Total	52	100.0	99	100.0

Coagulation Time.—Inspection of Table XIV reveals that no significant difference in coagulation time between the control and the benzol groups was found to exist. In the two cases already mentioned as having prolonged bleeding time, retraction of the clotted blood was delayed and the clot remained friable

Erythrocyte Sedimentation Rate.—Included in the present studies for the sake of completeness is the erythrocyte sedimentation rate, which has been said to yield abnormal results in a great variety of diseased states. Table XV shows a very close agreement between the results in the control and benzol groups. It may be said that the sedimentation speed of the red corpuscles is probably of no significance in connection with benzol exposure.

TABLE XV

COMPARISON OF SEDIMENTATION RATES OF ERYTHROCYTES IN CONTROL AND BENZOL GROUPS

ERYTHROCYTE SEDI- MENTATION RATE (MM. PER HOUR)	CONTROL		BENZOL	
	NUMBER	PER CENT	NUMBER	PER CENT
Less than 9	51	86.5	93	89.4
More than 9	8	13.5	11	10.6
Total	59	100.0	104	100.0

Hematocrit (Cell Volume).—It is not surprising that in the benzol group where anemia was a frequent finding, the hematocrit values should show

tendency to be lower than in the control group. Table XVI shows that in the benzol group nearly twice as many individuals were found with cell volumes less than 45 per cent as in the control group, and conversely, twice as many in the control group had values greater than 45 per cent. This difference is probably nothing more than a function of the anemia and has no specificity as far as benzol exposure is concerned.

TABLE XVI

DISTRIBUTION OF HEMATOCRIT VALUES IN CONTROL AND BENZOL GROUPS

HEMATOCRIT (CELL VOLUME %)	CONTROL		BENZOL	
	NUMBER	PER CENT	NUMBER	PER CENT
Less than 40	1	1.2	16	15.4
40-44	31	38.4	59	56.7
45-49	47	58.0	27	26.0
50 or more	2	2.4	2	1.9
Total	81	100.0	104	100.0

Mean Corpuscular Volume (M.C.V.)—As was previously mentioned the mathematical method of measuring the average size of the erythrocytes was considered more suitable for the present study than methods depending on estimates or measurements of cell size in stained blood films. (M.C.V. = hematocrit $\times 10 \div$ red blood cells in millions.) Apparently this is the first time this method has been applied to a large group of individuals who have been subjected to exposure of the type described in this study. Since the test is simple and well standardized, comparison of subsequent studies with those reported here can reasonably be made.

TABLE XVII

DISTRIBUTION OF MEAN CORPUSCULAR VOLUME VALUES (AVERAGE SIZE OF ERYTHROCYTES) IN CONTROL AND BENZOL GROUPS

MEAN CORPUSCULAR VOLUME (μ)	CONTROL		BENZOL	
	NUMBER	PER CENT	NUMBER	PER CENT
70-74	7	9.3	1	0.9
75-79	9	12.0	2	1.9
80-84	19	25.4	9	8.8
85-89	20	26.7	19	18.5
90-94	13	17.3	24	23.2
95-99	3	4.0	15	14.6
100-104	4	5.3	16	15.5
105-109	0	0	8	7.8
110-114	0	0	7	6.9
115-119	0	0	2	1.9
Total	81	100.0	103	100.0

The normal range for mean corpuscular volume given by Wintrobe⁹ is from 80 to 94 μ . In the present study 69.4 per cent of the patients in the control group fall within this range, with 21.3 per cent below and 9.3 per cent above. In the benzol group 50.5 per cent fall within Wintrobe's normal range, 2.8 per cent are below and 46.7 per cent are above. The details are given in Table XVII. No matter how the figures are analyzed, no conclusion seems possible except that patients in the benzol group had larger erythrocytes than those in the control group; in other words, the anemia associated with benzol exposure is of the

macrocytic type. The fact that the technique followed for determining mean corpuscular volume was a slight modification of that described by Wintrobe⁹ does not invalidate the comparison between the control and benzol groups in this study.

Mean Corpuscular Hemoglobin (M.C.H.).—This method of expressing the average hemoglobin content of the erythrocytes is analogous to color index. It is the preferred method since it is not influenced by varying views as to what constitutes 100 per cent hemoglobin or 100 per cent red corpuscles. (M.C.H. = grams of hemoglobin per 1,000 c.c. of blood \div red blood cells in millions.)

According to Wintrobe,⁹ the upper limit of normal for M.C.H. is 32 μg . Table XVIII shows that no case in the control group exceeded this figure, whereas 30.9 per cent of the benzol group had abnormally high M.C.H. values. Further, in the low ranges, 24 to 29 μg , there were found 87.7 per cent of the controls and only 35.8 per cent of the benzol cases. The findings seem to indicate quite definitely that there is hyperchromia of the erythrocytes of individuals who have been exposed to benzol.

TABLE XVIII

DISTRIBUTION OF MEAN CORPUSCULAR HEMOGLOBIN VALUES (AVERAGE HEMOGLOBIN CONTENT OF ERYTHROCYTES) IN CONTROL AND BENZOL GROUPS

MEAN CORPUSCULAR HEMOGLOBIN (μg)	CONTROL		BENZOL	
	NUMBER	PER CENT	NUMBER	PER CENT
24-26	30	41.2	12	10.0
27-29	34	46.5	31	25.8
30-32	9	12.3	40	33.3
33-35	0	0	21	17.5
36-38	0	0	11	9.2
39-41	0	0	5	4.2
Total	73	100.0	120	100.0

TABLE XIX

DISTRIBUTION OF MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION VALUES IN CONTROL AND BENZOL GROUPS

MEAN CORPUSCULAR HEMOGLOBIN (CONC. PER CENT)	CONTROL		BENZOL	
	NUMBER	PER CENT	NUMBER	PER CENT
25-26	1	1.2	0	0
27-29	5	6.1	2	1.9
30-32	55	67.9	40	38.0
33-35	19	23.6	53	50.6
36-38	1	1.2	9	8.6
39	0	0	1	0.9
Total	81	100.0	105	100.0

Mean Corpuscular Hemoglobin Concentration (M.C.C.).—The purpose of performing the mean corpuscular hemoglobin concentration is to ascertain whether hyperchromia is merely a reflection of macrocytosis, or whether it is a result of actual overloading of cells with hemoglobin. (M.C.C. = grams of hemoglobin per 100 c.c. of blood \div hematocrit. The result is expressed in per cent.) Wintrobe⁹ gives 33 to 38 per cent as the normal range, this being slightly

higher than that of the control group in the present study. If one compares the benzol group with the controls (Table XIX), it is apparent that the great majority in each group fell between 30 and 35 per cent; 91.5 per cent of the controls and 88.6 per cent of the benzol group were in this range. Only one individual in the entire series had an M.C.C. value above Wintrobe's normal limit. Nevertheless, the trend in the benzol group is distinctly higher than that of the controls, and statistical analysis of the two groups (see Table XXII) indicates that there is a significant difference. This would suggest that the hyperchromia associated with benzol exposure is due not only to macrocytosis but also to increased concentration of pigment in the erythrocytes.

Fragility of the Erythrocytes.—The test for fragility of the erythrocytes was performed on 68 controls and 92 members of the benzol group. When the results in the two groups were compared, no significant difference was found. This being the case, the findings are not given in detail.

Serum Bilirubin.—Since the test for serum bilirubin was not performed in the control group, the normal values given by the authors of the method followed¹⁰ will have to serve for comparison. These observers state that a serum bilirubin value greater than 0.75 mg. per 100 c.c. of blood is abnormal. Table XX shows that 32.4 per cent of the workers in the benzol group exceeded this figure, with 10.8 per cent having more than 1.0 mg. per cent.

TABLE XX
DISTRIBUTION OF SERUM BILIRUBIN VALUES IN THE BENZOL GROUPS

SERUM BILIRUBIN (MG. PER CENT)	BENZOL	
	NUMBER	PER CENT
Less than 0.75	69	67.6
0.75-1.0	22	21.6
More than 1.0	11	10.8
Total	102	100.0

Patients Showing Severe Hematologic Abnormality.—In order that some comparison might be made between the present series and those cases which form the basis for most of the reports in the literature, a group of individuals showing severe hematopoietic damage has been selected for special consideration. The group was selected arbitrarily and consists of cases in which any one of the following conditions was present: 1. Erythrocytes of 3.0 million or less. 2. Leucocytes of 4,000 or less. 3. Platelets of 50,000 or less.

A composite picture of the more important findings in this group is given in Table XXI. Since these cases have been artificially selected, any extensive analysis of the blood pictures would have little meaning. Among other things, Table XXI reveals the following blood findings:

1. Every case but one showed some degree of leucopenia. In 3 cases (Nos. 9, 12, and 20) the leucopenia was not associated with significant diminution in erythrocytes or thrombocytes.
2. Fifteen of the 23 cases had subnormal red blood cell counts. In all but two of these the white blood cells and platelets were also distinctly subnormal.

TABLE XXI
BLOOD FINDINGS IN THE SEVERELY INVOLVED GROUP

CASE NO.	Hb. (GM./100 C.C.)	R.B.C. (MILLIONS)	W.B.C.	LYMPH. (%)	PLATE-LETS	BLEEDING TIME (MIN.)	COAG. TIME (MIN.)	HEMATO-CRIT (C.V. %)	M.C.V.	M.C.H.	M.C.C.	E.S.R.	SERUM BILIRUBIN (MG. %)
1	13.7	3.51	4,650	34	49,000	2	11	40	114	39	34	25	0.84
2	13.2	3.97	3,800	28	159,000	1	8	41	104	33	32	2	1.02
3	15.1	5.19	3,750	28	62,000	1	8	41	79	29	37	4	0.93
4	11.7	2.94	2,650	29	38,000	0.5	9	30	103	40	39	1	1.12
5	8.0	2.49	3,900	34	27,000	1	7	24	97	32	33	4	1.03
6	13.8	4.73	4,550	32	46,000	15	11	42	89	29	33	1	0.93
7	11.7	3.11	3,300	36	28,000	2	7	34	111	38	38	2	---
8	12.7	5.08	4,750	25	50,000	2.5	6	42	83	25	30	1	0.73
9	14.1	4.59	3,750	35	152,000	1.5	5	47	101	31	30	1	0.73
10	13.7	3.69	4,200	24	14,000	6	---	38	104	37	36	2	---
11	11.2	3.20	3,100	36	59,000	2	8.5	31	97	35	36	16	0.87
12	14.1	4.98	3,700	23	149,000	---	---	---	---	29	---	---	---
13	14.5	4.24	3,150	36	34,000	4	12	46	109	34	31	6	0.57
14	12.6	3.90	4,000	53	43,000	3.5	15	36	92	32	35	6	0.63
15	14.8	4.56	5,050	41	50,000	2.5	12	46	101	32	32	1	0.97
16	11.4	2.50	2,750	42	30,000	15	11	30	120	46	38	4	0.90
17	15.2	4.59	5,400	42	14,000	2.5	15	41	89	33	37	6	0.66
18	15.3	4.06	4,150	37	36,000	1.5	9.5	46	113	38	33	10	0.68
19	12.7	3.98	3,700	34	56,000	3	11.5	36	90	32	35	2	0.71
20	14.6	4.63	3,900	29	133,000	3	11	42	91	32	35	1	0.71
21	15.1	4.26	11,000	24	26,000	2	10	45	105	35	33	1	0.63
22	13.0	4.47	3,200	30	121,000	1	10	40	90	29	33	2	0.48
23	13.4	3.74	3,800	34	75,000	1	9	40	107	35	33	2	0.52

TABLE XXII

STATISTICAL COMPARISON OF THE CONTROL AND BENZOL GROUPS

The mean values were calculated from frequency distributions and hence vary slightly from those in Table IV, which were calculated directly. The $\frac{x}{\sigma}$ values may be considered to indicate significant differences in all instances.

	CONTROL					BENZOL					DIFF. IN MEANS	σ OF DIFF. IN MEANS	DIFF. $\frac{x}{\sigma}$ OF DIFF.
	NO. OF CASES	MEAN	σ	σ OF MEAN		NO. OF CASES	MEAN	σ	σ OF MEAN				
Hemoglobin	81	14.34	0.7928	0.0881		235	13.87	1.07	0.0698		0.47	0.1122	4.18
R.B.C.	81	5.32	0.4239	0.0471		139	4.48	0.6681	0.530		0.84	0.0707	11.88
W.B.C.	81	8.253	1.896	211		332	7.295	2.368	130		988	247.8	3.86
Lymph. %	81	35.25	11.65	1.29		112	31.79	9.65	0.9128		3.46	1.5838	2.18
Lymph. no.	81	31.12	1.136	126		112	29.06	846	80.0		1.406	149.3	7.4
Platelets	50	208,000	48,590	6,872		107	157,617	73,971	7,150		70,383	9,918	7.09
M.C.V.	81	85.83	7.24	0.8044		103	95.31	9.40	0.9262		9.68	1.3267	7.89
M.C.H.	73	27.6	2.016	0.236		120	31.6	3.759	0.342		4	0.416	9.62
M.C.C.	81	32.02	1.48	0.164		105	33.63	2.07	0.182		1.61	0.247	6.34

3. Thrombocytopenia was present in 18 of the 23 cases. In two of these (Nos. 15 and 17) the erythrocytes and leucocytes were not significantly reduced.

4. The erythrocytes were abnormally large in 14 of the 22 cases in which size was determined.

5. Only one of the 22 cases with leucopenia had even a slight elevation of the lymphocyte percentage.

Actually the lymphocyte figures were lower in the severely involved group than in the control group. Values above 40 per cent occurred in 35.7 per cent of the latter as compared with 17.4 per cent of the former.

DISCUSSION

It would seem superfluous to indulge in any extended discussion of the foregoing findings. Points of interest to the industrial hygienist have received attention in another publication⁴ which deals with benzol poisoning as an industrial hazard.

Certain statistical analyses of the results were undertaken. These are summarized in Table XXII. The analyses consisted in comparing the controls with the benzol group to see whether significant differences existed. The data reveal that in all instances in which the results were tested the differences between the two groups can be considered significant. It should be borne in mind that in this comparison the entire benzol group, and not selected cases, was used.

SUMMARY AND CONCLUSIONS

1. Complete blood studies were performed on a relatively large group of individuals who had been exposed to concentrations of benzol vapor ranging from 11 to 1,060 p.p.m. for periods of from six to sixty months.

2. For comparison, similar studies were performed on a similar group of workers in a nonhazardous industry.

3. The abnormalities most frequently observed among the workers exposed to benzol were (a) anemia, (b) macrocytosis, and (c) thrombocytopenia.

4. Leucopenia was present in only a small percentage of the men who had been exposed to benzol.

5. Relative lymphocytosis was not found.

6. Comparison of the benzol group with the controls indicated that benzol exposure is likely to result in both relative and absolute lymphopenia.

7. Neutropenia was relatively rare; monocytosis, eosinophilia, and basophilia were not found in the benzol group.

8. Prolongation of the bleeding time following benzol exposure was rare in this study and did not parallel the reduction in thrombocytes.

9. Prolongation of the coagulation time was rare in the benzol group.

10. Relatively high hemoglobin values were a common finding in the benzol group. This abnormality was associated with macrocytosis as well as with overloading of red corpuscles with pigment.

11. The erythrocyte sedimentation rate is apparently not altered in benzol exposure.

12. The fragility of the erythrocytes does not seem to be significantly altered in benzol exposure but there seems to be a slight elevation of the serum bilirubin.

The studies covered in this paper were made while I was a member of the staff of the Division of Industrial Hygiene of the New York State Department of Labor. Through the courtesy of Dr. Leonard Greenburg, Executive Director of the Division of Industrial Hygiene, the data were made available for this report. Invaluable assistance in the statistical analyses was given by Dr. Morton Kramer, of the New York State Department of Health.

REFERENCES

1. Santesson, C. G.: Ueber chronische Vergiftung mit Steinkohlentheerbenzin, Vier Todesfalle, Arch. f. Hyg. 31: 336, 1897.
2. Toxicity of Industrial Organic Solvents, Medical Research Council, Industrial Health Research Board, London, 1937, H. M. Stationery Office.
3. Hamilton, A.: Benzene (Benzol) Poisoning, Arch. Path. 11: 434, 601, 1931.
4. Greenburg, L., Mayers, M. R., Goldwater, L., and Smith, A. R.: Benzene (Benzol) Poisoning in the Rotogravure Printing Industry in New York City, J. Indust. Hyg. & Toxicol. 21: 395, 1939.
5. Erb, L. A., and Rhoads, C. P.: The Hematological Effects of Benzene (Benzol) Poisoning, J. Indust. Hyg. & Toxicol. 21: 421, 1939.
6. Schilling, V. (Trans. by Gradwohl, R. B. H.): The Blood Picture and Its Clinical Significance, 7th and 8th revised editions, St. Louis, 1929, The C. V. Mosby Co.
7. Todd, J. C., and Sanford, A. H.: Clinical Diagnosis by Laboratory Methods, ed. 8, Philadelphia, 1937, W. B. Saunders Co.
8. Wintrobe, M. M., and Landsberg, J. W.: A Standardized Technique for the Blood Sedimentation Test, Am. J. M. Sc. 189: 102, 1935.
9. Wintrobe, M. M.: The Size and Hemoglobin Content of the Erythrocyte, J. LAB. & CLIN. MED. 17: 899, 1932.
10. Ernst, Z., and Förster, J.: Ueber die Bestimmung der Blutbilirubin, Klin. Wchnschr. 3: 2336, 1924; Quoted by Soffer, L. J.: Bull. Johns Hopkins Hosp. 52: 365, 1933.
11. Osgood, E. E.: Normal Hematologic Standards, Arch. Int. Med. 56: 849, 1935.
12. Osgood, E. E., Brownlee, I. E., Osgood, M. W., Ellis, D. M., and Cohen, W.: Total, Differential and Absolute Lymphocyte Counts and Sedimentation Rates Determined for Healthy Persons Nineteen Years of Age and Over, Arch. Int. Med. 64: 105, 1939.
13. Ref. 2, page 51.
14. Hunter, F. T.: Chronic Exposure to Benzene (Benzol). II. The Clinical Effects, J. Indust. Hyg. & Toxicol. 21: 331, 1939.
15. Hamilton, A.: Industrial Toxicology, New York, 1934, Harper & Brothers.

THE ARTERIAL CIRCULATION OF THE LOWER EXTREMITIES IN CHRONIC ARTHRITIS*

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A STUDY of the arterial circulation of the lower extremities in chronic arthritis was suggested by the repeated observation of vascular disturbances in patients attending the Arthritis Clinic. Such circulatory abnormalities, particularly vasomotor, have been mentioned occasionally in treatises on arthritis. Search of the literature reveals little attention devoted to the arterial circulation of the limbs in the rheumatic diseases. The need for investigation in this field was pointed out recently in the annual survey of publications on arthritis.¹

METHODS AND CRITERIA

The patients included in this series were suffering from arthritis and related conditions for which they were subjected to the routine physical and laboratory examinations of the clinic. They were then referred to the vascular department for study of the arterial circulation of their lower extremities. Every patient was examined by both of us. The diagnosis of arthritis or related condition was based on physical examination, laboratory data, such as total blood count, non-filament count, sedimentation rate, uric acid content of the blood, and roentgen examination. The diagnostic designations were rheumatoid arthritis, active or inactive; typical or atypical. Other classifications according to symptoms and signs were osteoarthritis, combined arthritis (osteoarthritis and rheumatoid), fibrositis, gout, and sciatic syndrome.

The criteria for the diagnosis of arterial disturbance were, in the order of importance: plantar ischemia, relative coldness of the feet, reduced oscillometric readings at the pedal and ankle levels, and diminished amplitude or absence of palpable arterial pulsation. Roentgen evidence of calcified arteries of the legs and feet was also taken into consideration. Plantar ischemia was sought after elevation and repeated flexion of the feet, according to the method described by one of us (S. S. S.). Rubor on lowering the extremities is not constant in incipient arterial disease but was noted when present. Relative coldness of the feet was estimated by a thermocouple and by palpation. Posterior tibial and dorsalis pedis pulses were palpated for their presence and amplitude. Pulse tracings of the feet and legs were made with a Tycoos recording oscillogram.

It is necessary to distinguish arteriosclerosis from arteriosclerosis obliterans. The former denotes structural changes in the walls of the arteries not, however, necessarily causing obstruction to arterial onflow. Calcification may be present to such a degree as to be visible in roentgenograms, but there may not be sufficient narrowing or blockage of the lumen of the arteries to cause diminished

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flow. However, when the element of obstruction is introduced by narrowing of the lumen or by actual blocking with a thrombus, the terminology as well as the physical conditions are changed, resulting in arteriosclerosis obliterans.

Unilateral arterial spasm occurs in varying degree in incipient organic arterial disease. As a rule, spasm merely accentuates existing changes produced by the organic lesion. In other words, it may intensify ischemia, it may increase coldness and may further diminish the amplitude of arterial pulsation. Any or all of these effects of spasm may be either abolished completely or modified by injection of novocain about the posterior tibial nerve. If ischemia, temperature, and pulse amplitude are affected by this injection, one must still consider the underlying condition an organic lesion because of its unilateral occurrence. Pure spasm in an isolated extremity is rare except in the case of direct external trauma to the large arteries of the limb.

For these reasons, the presence of plantar ischemia, coldness, and diminished or absent oscillometric pulsation either alone or in combination, with asymmetric distribution, has been considered definite evidence of arterial disease. The cause of the arterial obstruction has been ascribed in every such instance to arteriosclerosis obliterans because of the common prevalence of this disease. Thromboangiitis obliterans is excluded because of its characteristic clinical picture of incidence in young males with accompanying venous involvement. The absence of superficial migrating phlebitis in any of these cases lends further doubt to the diagnosis of thromboangiitis obliterans as the cause of arterial obstruction in any of the cases here reported.

DIFFERENTIAL POSTERIOR TIBIAL BLOCK

In those patients presenting evidence of arterial obstruction, posterior tibial block, as described by Scott and Morton,³ was performed. The procedure proved helpful in doubtful diagnostic situations. Anomalous response occurred in two patients, in one of whom the temperature rose without any accompanying increase in arterial pulsation; in the other an increased oscillometric reading was registered following the block, but the temperature rose only slightly. In addition to color and temperature observations, oscillometric pulse records were made before and after blocking.

FINDINGS

As Table I indicates, there were 47 patients with rheumatoid arthritis, 71 with osteoarthritis, nine with fibrositis, two with gout, two with gonorrheal arthritis, four with mixed arthritis, four with sciatica. The average age in the rheumatoid group was 44.4 years; in the osteoarthritis group, 51.3 years.

Plantar ischemia on elevation of the lower extremities, with or without brief flexion of the feet, was observed in 26 patients with rheumatoid arthritis, and in 22 patients with osteoarthritis. In a few patients with rheumatoid arthritis accompanied by vasomotor disturbances some plantar blanching was noticed in the normal position.

Palpable coldness, or lowered skin temperature, was noted in 26 rheumatoid patients and in 22 osteoarthritic patients. These observations confirm previous reports of the surface temperature changes in these diseases. The tem-

perature abnormalities were determined by thermocouple readings. It was found that gross estimation of temperature differences in the two extremities by the fingers of a trained observer were as reliable as instrumental readings in about 80 per cent of the cases.

TABLE I

TYPE OF ARTHRITIS	RHEU- MATOID	OSTEOAR- THRITIS	FIBRO- SITIS	GOUT	G.C.	MIXED	SCI- ATICA
Average age	44.4 Yr.	51.3 Yr.					
Paresthesia	5	5	0	1		1	11
Intermittent Claudication	2	1	0	1		0	
Coldness	28	28	1	1	1	2	2
Cyanosis	10	11	0	1		0	
Rubor	15	5	0	1		0	
Ischemia	24	23	1	1	1	4	2
Temperature changes	26	22	1	1		2	2
Pulsation absent	9	8	1	0		0	1
Oscillometer reading abnormal	22	18	1	2		0	2
X-rays	18	20	1	2		2	2
Total patients	47	71	9	2	2	4	4
Arteriosclerosis obliterans	3	2	1	1	0	0	1
Spasm	28	23	0	1	0	3	0
Negative	(59.5%)	(33%)					
Arteriosclerosis obliterans %	6.3%	2.8%	8	0	2	1	3
Total vascular disturbances	65.9%	35.2%					

Oscillometric readings were diminished in 22 patients with rheumatoid arthritis and in 18 with osteoarthritis. The amplitude of individual oscillations was important in determining any abnormal variation. Differences in readings at corresponding levels of both extremities, as illustrated in Figs. 1 and 2, were considered of great diagnostic importance.

Pulsations of one or more arteries were absent in nine patients with rheumatoid arthritis and in eight with osteoarthritis. Pulsations of the dorsalis pedis and of the posterior tibial arteries were studied. While an effort to determine the presence and the force of these pulses was made, this information was considered to have little, if any, diagnostic value in incipient arterial disease. Color changes on lowering the extremities were found in a small number of patients. Cyanosis of the feet was observed in ten patients with rheumatoid arthritis and in 11 with osteoarthritis. Rubor was noted in 15 patients with rheumatoid arthritis and in five with osteoarthritis.

Symptoms indicating intermittent claudication, despite the high incidence of circulatory abnormality, were elicited in only two patients with rheumatoid arthritis and in one with osteoarthritis.

Paresthesias of the lower extremities were described by five patients with rheumatoid arthritis and five with osteoarthritis. These symptoms are interesting and present a diagnostic problem in arthritis with arterial disturbance because of the difficulty of relegating them to either a circulatory or an arthritic basis.

In accordance with the above findings, as indicated in Table I, 28 patients with rheumatoid arthritis were considered as presenting evidence of vasospastic arterial disturbance; 23 patients with osteoarthritis showed signs of a similar condition. Three patients with rheumatoid arthritis gave conclusive evidence of arteriosclerosis obliterans, while two patients with osteoarthritis

appeared in the same category. When the findings were suggestive, but not definitely indicative of occlusion, the case was placed in the vasospastic group. There were eight such cases.

In a group of nine patients with fibrositis one showed signs of arteriosclerosis obliterans. In none of the others was there demonstrable evidence of arteriospasm. Of four patients with combined arthritis three had varying degrees of arteriospasm. One of four patients with sciatica showed arteriosclerosis obliterans; in three there were no vascular abnormalities. Of two patients with gout there were signs of arteriosclerosis obliterans in one and of arteriospasm in the other. Two patients with gonorrheal arthritis showed no circulatory disturbances.

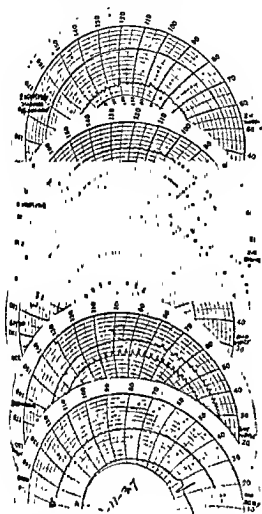


Fig. 1.

Fig. 1.—Reduced and unequal pedal pulses in arthritis. Each tracing shows both pedal pulses in the same patient.

Fig. 2.

Fig. 2.—Reduced and unequal tibial pulses in arthritis. Each tracing shows both tibial pulses in the same patient.

In our survey of 139 patients with rheumatic disease, therefore, seven presented signs of arteriosclerosis obliterans. Insufficient or no evidence for diagnosis of arterial disturbance occurred in 16 of 47 patients with rheumatoid arthritis, and in 47 of 71 patients with osteoarthritis.

In the series as a whole, of 47 patients with rheumatoid arthritis, 28, or 59.5 per cent, showed signs and symptoms of vasospastic arterial disturbance. Of 71 patients with osteoarthritis, 23, or 32.3 per cent, gave evidence of a similar condition. In the rheumatoid group three, or 6.3 per cent, gave indications of arteriosclerosis obliterans, whereas two, or 2.8 per cent, with osteoarthritis, presented a similar complication.

Within the rheumatoid group of 47 patients, then, 31, or 65.9 per cent, gave clinical evidence of abnormality in the arterial circulation of the lower extremities, while of 71 patients with osteoarthritis 25, or 35.2 per cent, presented such findings. Several patients in other classifications indicated in Table I showed signs of arterial disturbances, but these groups were so small that the individual cases, although suggestive, will not be considered further in this discussion. One patient with rheumatoid disease and another with osteoarthritis suffered from gangrene of an extremity. In each of these, however diabetes mellitus was a pronounced complicating factor.

TABLE II
INCIDENCE OF INFECTIOUS ACTIVITY AND JOINTS INVOLVED IN ARTHRITIS
WITH ARTERIAL DISTURBANCES

	TOTAL PATIENTS	PATIENTS WITH ARTERIAL SIGNS	SITE OF ARTHRITIC SIGNS AND SYMPTOMS WITH ARTERIAL DISTURBANCES				UNILATERAL ARTERIAL DISTURBANCES
			GENER- ALIZED	KNEES AND FEET ONLY	SPINE	UPPER EXTREM- ITIES ONLY	
Rheumatoid:							
Active	30	23	17	1		5	8
Inactive	17	8	6	1		1	1
Osteoarthritis	71	25	9	10	5	1	7

RELATION OF PERIPHERAL ARTERIAL DISTURBANCES TO LOCATION OF ARTHRITIS

A natural question arising in the course of this investigation was the possibility of a relationship between circulatory signs and arthritic involvement in the same extremity. As shown in Table II, a limited local reaction in isolated extremities did appear in a small number of patients and suggested as one possible source of vasomotor symptoms a local reflex angiospasm perhaps as a reaction to the nearby painful joint. While this study was confined to recording the arterial signs in the lower extremities, striking vasomotor symptoms in the upper limbs of these patients were frequently seen.

The influence of rheumatoid disease upon the location of vasomotor manifestations is indicated in Table II. Of 23 patients with active rheumatoid arthritis presenting vasomotor disturbances of the lower extremities, 17 had arthritic symptoms that involved all extremities, five showed limitation of joint trouble to the upper extremities, and only one to the knees and feet. In eight persons with inactive but symptomatic rheumatoid disease and associated circulatory signs in the lower extremities, six showed generalized joint involvement and discomfort; one presented arthritic symptoms restricted to the knees and feet, and one had involvement of the upper extremities only. These observations indicate that the location of peripheral vasospasm in many of the patients is unrelated to the site of rheumatoid activity.

Of 71 patients with osteoarthritis, as listed in Table II, 25 showed arterial disturbances of the lower extremities. Only ten of this group presented arthritic complaints limited to the joints of the lower extremities.

The higher, almost twofold, incidence of vasomotor symptoms found in rheumatoid disease as compared with osteoarthritis raises the question as to whether varied causative factors account for the difference. Close analysis

reveals that circulatory symptoms are not as prevalent in rheumatoid arthritis when the infectious process has subsided. Table II shows that the frequency of arteriospastic signs was highest in active rheumatoid arthritis (23 of 30 patients, or 77 per cent), next highest among those with inactive rheumatoid arthritis (8 of 17 patients, or 47 per cent), and least (25 of 71 patients, or 35.2 per cent) in osteoarthritis.

It is tempting to attribute the high incidence of peripheral arterial symptoms in active rheumatoid disease to the irritative effect of the underlying systemic infection. Such an assumption, however, could not clearly explain similar circulatory symptoms in apparently inactive rheumatoid arthritis and certainly does not account for vasospastic complaints in osteoarthritis which is generally considered to have a degenerative rather than an infectious basis.

It is readily discerned that the one clinical feature all of the three groups of arthritis present in common is symptomatic, painful articular disease. After thorough consideration of these facts one, therefore, must deduce that in these joint conditions some close, dynamic relationship may exist between painful, arthritic symptoms and the peripheral vasomotor imbalance with which they are frequently associated. Such a relationship appears most commonly, it is true, in the presence of active rheumatoid disease in which it is probable that systemic infection alone often may produce the circulatory disturbances by a local, toxic neurovascular reaction or, more likely, by central stimulation of the vegetative nervous system. It is further possible that systemic toxins in active, rheumatoid arthritis may supplement and augment the reflex mechanism initiating arterial disturbances, presumably as in quiescent rheumatoid infection and in osteoarthritis. This does not include the majority of patients with symptomatic osteoarthritis who do not present vasomotor disturbances.

TABLE III

JOINT X-RAYS OF 87 PATIENTS WITH VASCULAR DISEASE

SEX										
M	F	10-20	21-30	31-40	41-50	51-60	61-70	71-80	81-	
70	17		1	6	22	28	26	4		
X-RAYS POSITIVE							HISTORY AND SIGNS POSITIVE			
RHEUMATOID ARTHRITIS		OSTEOARTHRITIS					NEGA-TIVE	POSITIVE	RHEU-MATOID ARTHRITIS	OSTEO-ARTHRITIS
Nega-tive	Posi-tive	Knees	Foot	Sh	Sp	H				
	1	26	8	1	1	1	58 67.6%	28 32%	4 4%	15 17%

ARTHRITIS IN PATIENTS WITH VASCULAR DISEASE

As a control of the vascular study in arthritis, a group of patients with evidence of long-standing arterial disease attending the vascular clinic were studied for history, complaints, signs, and roentgen evidence of arthritis. According to Table III, of 86 individuals in this series under treatment for frank arterial disease, 58, or 67.6 per cent, gave no information or signs of rheumatic involvement at any time. Twenty-eight patients, or 32 per cent, yielded positive histories, physical signs, or roentgen evidence of rheumatic or arthritic involvement in some form.

THERAPY IN ARTERIAL DISTURBANCES IN ARTHRITIS

A variety of medical^{4, 5} and surgical^{6, 7} measures have been suggested to improve the peripheral circulation in arthritis. Although this report embraces an essentially diagnostic investigation, mention of some therapeutic experiences may not be irrelevant. A limited group of patients with arthritis showing vasospastic or occlusive disturbances were subjected to intravenous hypertonic saline therapy. Of 12 patients treated from three to eighteen months, seven showed marked improvement in symptoms, particularly in relief of discomfort in the lower extremities and in their ability to walk longer distances without the usual pain. The known angiospastic influence of tobacco warrants restriction of smoking in arthritis with severe vasomotor symptoms, together with other special care recommended for those patients with signs of arterial occlusion.⁸ The accepted value of alcohol in circulatory disturbances makes it a worthy addition to therapy in arthritis⁹ when vasospastic complaints are present.

SUMMARY AND CONCLUSIONS

1. In a clinical investigation of rheumatoid arthritis, 65.9 per cent of the patients showed abnormalities, usually vasomotor disturbances, of the arterial circulation of the lower extremities; in osteoarthritis 35.2 per cent of the cases presented similar findings. Three rheumatoid patients, or 6.3 per cent, had simultaneous arteriosclerotic occlusive disease; two with osteoarthritis, or 2.8 per cent, presented evidence of arteriosclerosis obliterans.

2. Study of those patients with arthritis presenting arterial abnormalities showed no direct, consistent relationship between the location of the vascular signs and the site of the arthritic process. The evidence suggests that the vasomotor disturbances in chronic rheumatoid arthritis and related conditions may be attributed to some systemic reaction probably acting through the sympathetic nervous system, although in some cases a local irritative reflex vasospastic mechanism in the painful arthritic extremity cannot be ruled out.

3. In a control group of 86 patients with advanced organic arterial disease, 28, or 32 per cent, offered a history, physical signs, or roentgen changes of rheumatic or arthritic involvement at some time. These findings approximate the incidence of such ailments in similar age groups of individuals without arterial disturbances and contribute clinical evidence that pronounced arterial disease alone does not usually produce arthritic signs and symptoms.

REFERENCES

1. Hench, P. S.: The Present Status of Rheumatism and Arthritis: Review of American and English Literature for 1936, *Ann. Int. Med.* 2: 1089, 1938.
2. Samuels, S. S.: The Early Diagnosis of Thrombo-angiitis Obliterans: A New Diagnostic Sign, *J. A. M. A.* 92: 1571, 1929.
3. Scott, W. J. M., and Morton, J. J.: The Differentiation of Peripheral Arterial Spasm and Occlusion in Ambulatory Patients, *J. A. M. A.* 97: 1212, 1931.
4. Kovacs, J.: The Iontophoresis of Acetyl-beta-methylcholin Chlorid in the Treatment of Chronic Arthritis and Peripheral Vascular Disease, *Am. J. M. Sc.* 188: 32, 1934.
5. Fox, R. F., and Van Breeman, J.: Chronic Rheumatism, London, 1934, J. & A. Churchill.
6. Rowntree, L. G., and Adson, A. W.: Ramisection of Sympathetic Ganglia and Trunks in Chronic Infectious Arthritis, *Ann. Int. Med.* 4: 331, 1930.
7. Patterson, R. H., and Stainsby, W. J.: The Therapeutic Effects Following Interruption of the Sympathetic Nerves, *Ann. Surg.* 103: 514, 1936.
8. Samuels, S. S.: The Diagnosis and Treatment of Diseases of the Peripheral Arteries. New York and London, 1940, Oxford University Press.
9. Weissenbach, R. J.: Use of Wine in Arthritis, *J. de méd. de Bordeaux* 110: 523, 1933.

EPINEPHRINE OUTPUT FROM THE ADRENAL GLANDS IN EXPERIMENTAL HYPERTHYROIDISM*

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IT IS often assumed that changes in irritability of the sympathetic nervous system that occur in hyperthyroidism are related to a corresponding increase in the rate of liberation of epinephrine from the adrenal glands. The investigation reported here was undertaken to obtain quantitative information on this question.

A significant role in the etiology and pathology of hyperthyroidism and of a number of other diseases, notably diabetes mellitus and arterial hypertension, is commonly attributed to the adrenal medulla. This is based chiefly on pharmacodynamic, sometimes toxic, reactions that are obtained on administration of epinephrine in pharmacologic doses. Support of quantitative measurements of the epinephrine output from the adrenals, under appropriate experimental conditions, is lacking.

Eppinger, Falta, and Rudinger¹ postulated a mutual stimulating interrelationship between the thyroid gland and the adrenal medulla. They suggested the probability that, in hyperthyroidism, the excess thyroid secretion may stimulate secretion of epinephrine from the adrenals, which in turn is responsible for increased irritability of the sympathetic nervous system. Such an interrelationship between the thyroid and adrenal glands seems unlikely, for, if it existed, a vicious cycle of mutually induced hypersecretion from both these glands might lead to accumulation of relatively large amounts of epinephrine and of thyroid hormone in the circulation.

We do not assume that experimentally created conditions are identical with similar conditions that occur clinically. Nevertheless, since certain of the pathologic manifestations often are attributed to an associated increase in the output of epinephrine from the adrenals, it seemed desirable to perform quantitative experiments for determination of the rate of liberation of epinephrine into the circulation in dogs subjected to prolonged thyroid feeding. These experiments are part of an investigation on possible functional interrelationships between the adrenals and the thyro-parathyroid apparatus. Experiments related to the parathyroid will be reported in another paper.

Six adult dogs, three males and three females, received desiccated thyroid (Armour's), represented to contain 0.2 per cent iodine, for periods ranging from three to twelve and a half weeks. The dosage of thyroid ranged from

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0.5 Gm. to 1.3 Gm. per kilogram of body weight, administered daily together with the regular food. The routine diet consisted of a mixture of boiled beef lung, white bread, and bone meal.

Water intake and urine output were measured daily. Pulse rate and body weight were recorded at frequent intervals, and in four of the animals observations of the rectal temperature were made from time to time. In three dogs, one of which served as a control, observations of the basal metabolic rate were made as an indication of the degree of experimental hyperthyroidism that was induced.

Kunde² has demonstrated that thyroid feeding, in doses and for periods comparable with those of our experiments, induces a hyperthyroid condition in dogs. Our determinations of the basal metabolic rate were made essentially according to the method employed by Kunde. The dogs, previously trained to lie quietly, were fasted for eighteen to twenty hours. Each observation was preceded by a preliminary "rest" period. A specially devised mask was fitted to the animal and was connected with a Benedict-Roth closed circuit apparatus for determination of oxygen consumption. At the conclusion of each observation the rectal temperature and pulse rate were recorded. Observations of the basal metabolic rate were made approximately four times weekly throughout the course of the experiment.

After a sufficient number of observations were made to determine a base line for each factor, administration of thyroid was begun. At the end of the period of thyroid feeding the animals were sacrificed for determination of the rate of liberation of epinephrine from the adrenals. The epinephrine output was measured by the method of Stewart and Rogoff, obtaining adrenal vein blood at a known rate of flow, via the "cava pocket," and determining the epinephrine concentration in the blood by its action on rabbits' intestine segments. Ether anesthesia was employed for the operative procedure.

All the epinephrine assays were performed by the same individual (R. C.). Epinephrine output was determined in eleven normal dogs for comparison with the experimental animals, under the same laboratory conditions. These are included in Table I. The results obtained compare very well with those in the large series of animals reported by Stewart and Rogoff.³

Table II illustrates the results obtained in the experiments on thyroid-fed dogs. Definite loss in body weight was observed in all animals, the largest percentage loss having occurred in the two dogs (Nos. 19, 25) that were subjected to thyroid feeding for the longest periods. For convenience, the figures given in the table for heart rate, rectal temperature, and basal metabolic rate are the averages for the control period and for the period of thyroid feeding. It may appear from the averages that differences between the figures for observations before and during the period of thyroid feeding seem insignificant. However, the actual changes that occurred were generally large enough to leave no doubt about the genuineness of the results.

Thus, in dog 12, while the average temperature during the period of thyroid feeding does not appear to differ from that during the control period, actually it had reached 102.7° F. at the time that the animal was sacrificed for determination of the epinephrine output from the adrenals. A similar gradual increase

TABLE I
EPINEPHRINE OUTPUT IN CONTROL DOGS

RECORD NUMBER	SEX	BODY WEIGHT (KG.)	ADRENAL WEIGHT (GM.)		EPINEPHRINE OUTPUT PER MINUTE (MG.)	
			RIGHT	LEFT	PER DOG	P
A	M	7.9	0.49	0.49	0.00056	0
B	M	8.1	---	---	0.00097	0
C	M	5.0	---	---	0.00055	0
D	M	11.5	0.62	0.75	0.0029	0
E	F	9.7	0.95	1.02	0.00087	0
F	M	12.9	0.55	0.53	0.00338	0
G	M	8.6	0.51	0.51	0.00198	0
H	F	6.5	0.51	0.51	0.0024	0
I	M	8.9	0.60	0.51	0.0007	0
J	M	8.3	0.45	0.56	0.00199	0
K	M	8.2	0.39	0.40	0.00139	0

TABLE II
EPINEPHRINE OUTPUT IN DOGS WITH EXPERIMENTAL HYPERTHYROIDISM

RECORD NUMBER	SEX	BODY WEIGHT (KG.)		THYROID FEEDING		AVERAGE HEART RATE PER MINUTE		AVERAGE TEMPERATURE		AVERAGE B.M.R. CAL./KG./HR.		POLYURIA	EPINEPHRINE OUTPUT PER MINUTE (MG./KG.)
		INITIAL	FINAL	AVERAGE DAILY DOSE (GM./KG.)	DURATION (WEEKS)	BEFORE FEEDING PERIOD	DURING FEEDING PERIOD	BEFORE FEEDING PERIOD	DURING FEEDING PERIOD	BEFORE FEEDING PERIOD	DURING FEEDING PERIOD		
3	M	13.3	11.0	0.5	6	54	76					0	0.00016
6	F	5.1	4.5	1.0	3	66	96					++	0.00014
10	F	10.7	9.8	1.3	6.5	84	88	101.8	102.2			++	0.00013
12	M	9.9	8.6	1.1	5.5	70	76	101.6	101.7			+	0.00013
19	M	11.2	8.6	0.9	12.5	53	80	100.5	101.3	1.51	2.48	0	0.00013
25	F	7.3	5.7	1.1	12.5	71	83	100.3	101.4	1.71	2.54	++	0.00010

was observed in heart rate in all the animals. Water intake and urine output were increased in four of the animals. The two dogs (Nos. 19, 25) in which observations of the basal metabolic rate were made showed an average increase of 61 per cent and 49 per cent, respectively, during the period of thyroid feeding.

The rate of liberation of epinephrine from the adrenals, in the animals with experimental hyperthyroidism, was not significantly different from that of the control, normal dogs. It was well within the range of epinephrine output usually observed in normal animals when measured directly by this method. In a small number of preliminary experiments on dogs, subjected to thyroid feeding for shorter periods, one of us (J. M. R.) obtained similar results.

In four cats, studied by Mr. George N. Stewart, prolonged thyroid feeding led to marked loss of weight in two, moderate loss in one, and no loss in one. In all of the animals, at the end of the experimental period, he found the epinephrine output from the adrenals very close to the average for normal cats, as determined by the same method.

Our experiments indicate that thyroid feeding does not cause a significant change in the rate of liberation of epinephrine from the adrenals, even when it produces a definite hyperthyroid state in the experimental animal.

DISCUSSION

Recent experimental investigations support the concept of functional interrelationship between various endocrine organs. This is especially true concerning interdependence between the anterior hypophysis and certain other glands. Evidence in support of a hypothetical interrelationship of a mutual character between two glands is incomplete unless there is provision for maintenance of a physiologic balance between the interrelated functions. Existence of an interrelationship limited to mutual stimulation (or depression) alone, without a control mechanism to limit or prevent continued and increasing overactivity (in either direction) of the related organs, appears to us as unlikely.

In the case of thyroid-adrenal interrelationship, a mutual stimulating action is believed to exist. The literature, largely clinical, generally supports this view. The experimental evidence in support of this hypothesis, however, is susceptible to serious criticism: it is not quantitative; attempts to measure epinephrine in blood were made with inadequate methods; indirect, nonspecific reactions have been relied upon as indications of increased epinephrine output from the adrenals.

In blood serum of individuals with exophthalmic goiter, demonstrable increase in epinephrine was reported by Kraus and Friedenthal,⁴ Fraenkel,⁵ and Bröking and Trendelenberg⁶ employing biological test objects. On the other hand, Gottlieb,⁷ who tested plasma, was unable to obtain epinephrine reactions. O'Connor⁸ demonstrated that the positive reactions were artifacts due to development of substances in clotting blood which gave reactions similar to epinephrine on the test objects.

Improper interpretation of such reactions may lead to serious error. Thus, Fraenkel,⁵ obviously having employed rabbits' uterus segments without adequate control observations, concluded from the reactions obtained that serum

from systemic blood of normal individuals contains approximately 1:400,000 epinephrine, and that corresponding serum from patients with Basedow's disease contains four to eight times this amount. Arithmetical calculation indicates that he was misled by his tests. A concentration of 1:400,000 in the serum of systemic blood would correspond to approximately 1:2,000 in adrenal blood serum. Accordingly, in Basedow's disease concentrations corresponding to 1:500 to 1:250 adrenalin would be present in the adrenal vein blood serum, which seems highly improbable if not indeed impossible. The epinephrine concentration, reported by Fracnel, for normal systemic blood is about 200 or more times as great as that found by Rogoff and Marcus⁹ to be capable of effecting hemodynamic and other, sometimes toxic, reactions.

The tonus-increasing action on rabbit's uterus (and intestine) or on a vascular preparation (frog perfusion method) has been shown by Stewart¹⁰ to be a serum effect, obtainable with unclotted blood, serum, or plasma. Epinephrine, however, produces inhibition of tone and contractions on rabbit's intestine, and the opposite on the nonpregnant or virgin uterus. This fact, indeed, incorporated in the method developed by Stewart and Rogoff, constitutes an important advantage over any other method at present available for quantitative assay of epinephrine in blood. It permits the elimination of artifacts produced by substances (or conditions) other than epinephrine, which yield epinephrine-like reactions on the intestine, uterus, vascular preparations, or denervated structures.

With sensitive test objects, Stewart¹⁰ was unable to detect epinephrine in systemic blood obtained from a case of Grave's disease. Rogoff and Wasserman¹¹ obtained negative results in tests for epinephrine on systemic blood from patients with hypertension. Rogoff and Goldblatt¹² were unable to detect the presence of thyroid hormone in blood obtained from the thyroid vein during surgical operations for goiter; they employed the sensitive endpoint reaction in their tests.

Various attempts have been made to study the influence of thyroid extracts or thyroxin on the epinephrine output from the adrenal glands. None of these offer quantitative data. Ott and Scott¹³ injected crude thyroid extracts and tested vena cava blood obtained through a catheter inserted through the femoral vein. Zunz and La Barre¹⁴ performed crossed-circulation experiments in which intravenous injection of thyroxin was followed by determinations of the blood sugar level. They concluded that a gradual augmentation of epinephrine output occurs, which is associated with hyperinsulinemia caused by the thyroxin. Quantitative measurements of epinephrine output were not made.

Kuriyama¹⁵ found no evidence that the acidosis induced in rabbits by thyroid feeding is induced by hypersecretion of epinephrine. Results obtained by histologic examination of the adrenals, e.g., Herring,¹⁶ Cramer,¹⁷ cannot be relied upon as an indication of the amount of epinephrine liberated by the glands. Like observations on the epinephrine load of the glands, they can indicate only the balance between production and liberation and do not indicate the output.

Gley and Quinquaud¹⁸ obtained adrenal blood from the adrenal veins and studied its pressor effects when injected into the circulation of a test animal.

They found no significant difference between the effects of control specimens and specimens obtained after injection of thyroid extracts. Their experiments were the most direct but lack quantitative significance since they did not take account of possible changes in the rate of blood flow through the adrenals at the time of collection of the blood specimens.

In experiments where the observations were made shortly after administration of thyroid extract or thyroxin, it may be pointed out that thyroid effects could hardly have been expected. Kunde² demonstrated that changes in the basal metabolic rate do not occur until from seven to twelve hours after administration of thyroid. In our experiments thyroid feeding was continued for sufficiently long periods to cause definite thyroid reactions. Quantitative determinations of the rate of liberation of epinephrine from the adrenals were made with a method that has proved reliable over a period of many years.

Epinephrine output in the hyperthyroid animals was not different from that in normal, control dogs. If epinephrine secretion from the adrenal glands is at all related to the phenomena associated with hyperthyroidism, it appears more likely that under these conditions the sympathetic nervous system might become more sensitive to the action of epinephrine in the amounts normally liberated. This possibility is suggested by the observations of Asher and Flack¹⁹ and others, and by the Goetsch²⁰ clinical test for hyperthyroidism.

SUMMARY

1. Experimental hyperthyroidism was produced in dogs by feeding desiccated thyroid for periods ranging from three weeks to three months. At the end of these periods the animals were sacrificed for determination of the rate of liberation of epinephrine from the adrenal glands.

2. Quantitative measurements of the epinephrine output from the adrenals showed that there is no detectable change in the rate of liberation of epinephrine. The output was within the same range as is found in normal, control animals.

REFERENCES

1. Eppinger, H., Falta, W., and Rudinger, C.: Über die Wechselwirkungen der Drüsen mit innerer Sekretion, *Ztschr. f. klin. Med.* 66: 1, 1908.
2. Kunde, M. M.: Studies on Metabolism. VI. Experimental Hyperthyroidism, *Am. J. Physiol.* 82: 195, 1927.
3. Stewart, G. N., and Rogoff, J. M.: The Average Epinephrine Output in Cats and Dogs, *Am. J. Physiol.* 66: 235, 1923.
4. Kraus, F., and Friedenthal, H.: Ueber die Wirkung der Schilddrüsenstoffe, *Berl. klin. Wehnschr.* 45: 1709, 1908.
5. Fraenkel, A.: Ueber den Gehalt des Blutes an Adrenalin bei chronischer Nephritis und Morbus Basedowii, *Arch. f. exper. Path. u. Pharmacol.* 60: 395, 1909.
6. Bröking, E., and Trendelenberg, P.: Adrenalin nachweis und Adrenalinhalt des menschlichen Blutes, *Deutsche Arch. f. klin. Med.* 103: 168, 1911.
7. Gottlieb, R.: Experimentelles zur Theorie des Morbus Basedowii, *Deutsche med. Wehnschr.* 37: 2161, 1911.
8. O'Connor, J. M.: Ueber Adrenalinbestimmung im Blute, *München med. Wehnschr.* 58: 1439, 1911.
9. Rogoff, J. M., and Marcus, E.: Supposed Role of the Adrenals in Hypertension. An Experimental Investigation, *J. A. M. A.* 110: 2127, 1938.
10. Stewart, G. N.: The Alleged Existence of Adrenalin (Epinephrin) in Pathological Sera, *J. Exper. Med.* 15: 547, 1912; Testing for Epinephrin (Adrenalin) in Blood. Comparison of Plasma and Serum, *Ibid.* 16: 502, 1912.
11. Rogoff, J. M., and Wasserman, P.: Unpublished data.

12. Rogoff, J. M., and Goldblatt, H.: Attempt to Detect Thyroid Secretion in Blood Obtained From the Glands of Individuals With Exophthalmic Goiter and Other Conditions Involving the Thyroid, *J. Pharmacol. & Exper. Therap.* 17: 473, 1921.
13. Ott, I., and Scott, J. C.: The Action of Glandular Extracts Upon the Amount of Epinephrine in the Blood, *J. Pharmacol. & Exper. Therap.* 3: 625, 1912.
14. Zunz, E., and La Barre, J.: Action de la thyroxine sur l'adrenalino-secretion, *Compt. rend. Soc. de biol.* 110: 95, 1932.
15. Kuriyama, S.: The Influence of Thyroid Feeding Upon Carbohydrate Metabolism. III. The Acidosis in Experimental Hyperthyroidism and Its Relation to Epinephrine in the Blood and the Decrease of Liver Glycogen, *J. Biol. Chem.* 33: 215, 1918.
16. Herring, P. T.: The Effects of Thyroidectomy and Thyroid-Feeding Upon the Adrenalin Content of the Suprarenals, *Quart. J. Exper. Physiol.* 9: 391, 1916.
17. Cramer, W.: Fever, Infections and the Thyroid-Adrenal Apparatus, *Brit. J. Exper. Path.* 7: 95, 1926.
18. Gley, E., and Quinquaud, A.: Contribution a l'etude des interrelations humorales. I. Action de l'extrait thyroïdien et en general des extraits d'organes sur la secretion surrenale, *Arch. internat. de physiol.* 14: 152, 1914.
19. Asher, L., and Flack, M.: Die innere Sekretion der Schilddrüse und die Bildung des inneren Sekretes unter dem Einfluss von Nervenreizung, *Ztschr. f. Biol.* 55: 83, 1910.
20. Goetsch, E.: "Epinephrin Hypersensitiveness Test" in the Diagnosis of Hyperthyroidism, *Pennsylvania M. J.* 23: 431, 1920.

THE BLOOD SEDIMENTATION RATE IN HEALTHY GIRLS*

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SINCE the fundamental studies of Fåhræus in 1917, the erythrocyte sedimentation test has become a very commonly used tool of the physician.¹ Despite the many possibilities of error in execution and interpretation, its comparative value has not diminished.

Abnormal sedimentation rates have been described in patients suffering from acute and chronic infections, malignancy, endocrine disturbances, infarction, and allergy. Pregnancy and meteorologic conditions seem to cause an increase in the velocity of sedimentation.² On the other hand, simple catarrhal inflammations, functional disorders, focal infections, and skin diseases do not seem to influence this test.

Most studies published have been concerned with the variations of the blood sedimentation rate in disease. The references listing physiologic changes in health describe a small variation between the two sexes, and an insignificant change in old age. A recent study of the rate in healthy young people disclosed a range of 1.0 to 30.0 mm. in 95 per cent of the cases, with a mean of 10.0 mm. in forty-five minutes. When plotted, a skew curve resulted, with the 15 mm. rate in forty-five minutes as the upper limit in 80 per cent of all the cases.³ The Westergren pipette and the potassium oxalate method was used in this particular study.

In another examination of healthy men and women, weekly changes were found, but always within normal limits.⁴

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Normal limits for the blood sedimentation rate by the Westergren method are 1.0 to 3.0 mm. per hour for men, and 4.0 to 7.0 mm. per hour for women. The Cutler method assumes 2.0 to 10.0 mm. per hour as the average for women in health.^{5, 6}

In a further attempt to obtain more accurate data concerning the blood sedimentation rates in healthy people, a total of 360 presumably healthy girls were studied with this idea in mind.

TABLE I
BLOOD SEDIMENTATION RATES OF 327 HEALTHY GIRLS

E.S.R. MM./HR.	NUMBER OF GIRLS IN AGE GROUPS (YEARS)											TOTAL
	10	11	12	13	14	15	16	17	18	19	20	
2	0	0	4	1	0	1	0	0	0	0	0	6
3	0	0	1	3	1	1	2	1	0	0	1	10
4	0	1	5	4	6	4	2	0	1	1	1	25
5	2	4	4	9	3	5	4	2	4	0	2	39
6	1	4	2	8	6	5	2	4	0	0	2	34
7	5	3	9	2	14	4	7	0	3	2	3	52
8	4	1	4	6	3	2	2	1	1	1	1	26
9	3	0	3	1	5	1	1	0	1	2	0	17
10	2	1	2	4	5	3	6	3	1	0	3	30
11	2	2	3	3	1	6	1	1	1	0	3	23
12	3	0	4	3	2	2	9	1	1	0	2	27
13	3	1	1	4	0	0	1	0	0	0	1	11
14	0	0	1	0	0	1	1	0	0	0	1	4
15	0	0	0	0	1	1	0	0	0	0	1	7
16	0	0	0	3	2	1	0	0	0	0	0	1
17	0	0	0	1	0	0	0	0	0	0	0	4
18	0	0	0	0	0	1	0	2	0	0	1	1
19	0	0	0	1	0	0	0	0	0	0	0	2
20	0	0	1	0	1	0	0	0	0	0	0	2
21	0	0	1	0	0	0	0	0	1	0	0	0
22	0	0	0	0	0	0	0	0	0	0	0	1
23	0	0	0	1	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0	0
25	1	0	0	0	0	0	0	0	0	0	0	1

METHODS

Inasmuch as it has been shown that the erythrocyte sedimentation rate is reasonably the same by the methods of Linzenmeier, Westergren, and Cutler, the latter method, being available, was used.⁷

The 5 c.c. graduated tube was used, with 3 per cent sodium citrate as the anticoagulant. Necessary corrections were made for anemia. All the bloods were withdrawn between 10:00 and 11:00 A.M., and the tests were performed the same morning.

All the young women studied were between 10 and 20 years of age, and were born in the state of Vermont. Each girl was given the usual physical and laboratory examinations, which included routinely, serologic tests for syphilis and undulant fever, tuberculin test, chest x-ray film with serial retakes when indicated, and blood morphology examinations in all cases of increased sedimentation rates.

A definite attempt was made to rule out any hidden chronic infections as syphilis, tuberculosis, undulant fever, localized suppuration, and rheumatic fever. All these girls were followed for at least four months after the

determination of the original sedimentation rate to limit the possibility of any disease process appearing subsequently that may have been present at the time of the original examination.

No sedimentation rate was determined during a period of common cold, two days before or following menses, or when it was thought peripheral dental infection or tonsillitis might be present, despite previous statements that no appreciable elevation in rate is present in these conditions.⁸

RESULTS

There are included in this report the blood sedimentation rates of 327 presumably healthy girls. All these girls were known not to have been ill at any time within four months after the blood was withdrawn for the original test.

As can be seen from Table I, 92 per cent of these girls had sedimentation rates between the limits of 2 mm. and 13 mm. per hour, whereas the rates of 8 per cent of the girls ranged from 14 mm. to 25 mm. per hour. The median fell at 7 mm. per hour.

CONCLUSION

Normal variations of any test must be ascertained before such a test can be relied upon for diagnosis.

This study suggests that in young women the blood sedimentation rate is variable, and that about 10 per cent of healthy young women may have sedimentation rates of greater velocity than the average arbitrary range.

The diagnostic value, therefore, of the erythrocyte sedimentation rate must be guided by these normal variations.

REFERENCES

1. Fåhræus, R.: Suspension Stability of Blood, *Physiol. Rev.* 9: 241, 1929.
2. Hoverson, E. T., and Petersen, W. F.: Meteorologic Effect on Sedimentation Rate of Erythrocytes, *Am. J. M. Sc.* 138: 455, 1934.
3. Osgood, E. E., and Others: Total, Differential, and Absolute Leucocyte Counts and Sedimentation Rates of Healthy Adolescents, 15 to 18 Years of Age, *J. LAB. & CLIN. MED.* 24: 954, 1939.
4. Greisheimer, E. M.: Blood Sedimentation Test in Normal Men and Women, *Am. J. M. Sc.* 174: 338, 1927.
5. Cutler, J. W.: Graphic Method of Blood Sedimentation Test: Presentation of 1 c.c. Technique and Other Important Modifications and Suggestions, *Am. Rev. Tuberc.* 19: 544, 1929.
6. Cutler, J. W.: Practical Application of Blood Sedimentation Test in General Medicine: Observations Based Upon Approximately 5,000 Patients Over Period of 6 Years, *Am. J. M. Sc.* 183: 643, 1932.
7. Greisheimer, E. M., Treloar, A. E., and Ryan, M.: Inter-Relation of Cutler, Linzenmeier and Westergren Sedimentation Tests, *Am. J. M. Sc.* 187: 213, 1934.
8. Wintrobe, M. M.: Application and Interpretation of Blood Sedimentation Test in Clinical Medicine, *M. Clin. North America* 21: 1537, 1937.

CLINICAL CHEMISTRY

STUDIES IN DYSTROPHIA MYOTONICA*

V. CREATINE AND CREATININE EXCRETION†

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AN ABNORMALITY in the metabolism of creatine and creatinine has been reported in several of the primary myopathies, as well as in certain conditions which secondarily affect the skeletal muscles. This abnormality may be manifested in several ways: (1) The creatine content of the muscles may be subnormal. (2) The ability to retain ingested creatine may be decreased. (3) Even on a creatine-free diet there commonly occurs a creatinuria which, according to Milhorat and Wolff,^{9, 10} is an expression of the total mass of improperly functioning muscle rather than of the amount of muscle which has actually degenerated. (4) The excretion of creatine may be greatly augmented when glycine is administered orally. (5) If there are extensive muscular atrophy and degeneration, creatinine excretion, which is dependent upon the total muscle mass, may be considerably below normal. All these symptoms are observed to a marked degree in progressive muscular dystrophy.

The recent discovery at the Colorado General Hospital and Outpatient Clinic of a number of patients with dystrophia myotonica from five different families‡ has given an unusual opportunity for studies of this disease,^{18-20, 22, 23} which is apparently not so rare as is commonly believed (cf. Stevenson in Cecil's *Textbook of Medicine*²⁴). In the course of these studies it was decided to investigate the excretion of creatine and creatinine in some of the patients and to compare the findings with those in previous reports of this disease and of progressive muscular dystrophy.

The literature on the metabolism of creatine and creatinine in dystrophia myotonica is summarized in Table I. From this table the following observations may be made: (1) According to one investigator¹⁵ creatine phosphate in resting muscles is below normal and its breakdown during contraction is subnormal. (2) Creatine tolerance is decreased in some cases. (3) Creatinuria is slight to moderate in degree and may or may not be augmented by glycine adminis-

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†The results given in this paper for patients J. M. and N. P. were taken from a thesis presented by Robert C. Lewis, Jr., to the Graduate School of the University of Colorado in partial fulfillment of the requirements for the degree Master of Science, August, 1937. A preliminary report of the work on patients J. M. and N. P. was made at the meeting of the American Association for the Advancement of Science at Denver, June, 1937.

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‡A patient from a sixth family has been admitted to the hospital since these studies were made.

AUTHOR	PATIENTS		CREATININE EXCRETION* (GM./24 HR.)	IMPROVED CREATININE COEFFICIENT*†	CREATINE EXCRETION (GM./24 HR.)	EFFECT OF GLYCINE ON CREATINE EXCRETION	CREATINE TOLERANCE‡	REMARKS
	NO.	SEX						
Pemberton ¹⁶	1	F	0.270-0.374 (avg. 0.403)	—	—	—	—	
Burger ²	3	M	0.646-0.819	9.6-16.7	0.002-0.038	—	—	
Meyers	2	F	—	Low	None	—	—	
Morgulis and Young ¹⁴	1	M	0.717-0.725	—	0.353-0.562 (avg. 0.458)	—	Zero (ingested creatine recovered quantitatively as extra creatine and creatinine)	Creatine excretion increased by administration of meat protein; not affected by feeding 5.3 gm. of gelatin per day
Kostakow and Slauck ⁷	1	F	0.26-0.29	—	More than 0.02	No effect	—	
Slauck ²³	?	F	0.4 (approx. §)	—	0.03 (approx. §)	Glycine alone, no effect; glycine plus testicular extract, marked increase	Less than 5%	
D'Antona ⁴	3	M	0.986-1.512	—	Slight to 0.158-0.298	Increase in 2 of 3 cases	—	
Adams, Power, and Boothby ¹	1	F	0.85	20.1	0.03	Slight increase	—	
Kolb, Harvey, and Whitehill ⁶	2	M	—	—	Slight	—	Normal in one case; 50% in one case	Creatine excretion not affected by prostigmine or quinine
Millhorat and Wolff ¹³	1	F	—	—	Normal	—	Normal	
	5	M	0.715-1.965	13.4-31.7	0.000-0.174	No effect or only slight increase	36-100%. Decreased in one case following glycine therapy	
	1	F	1.092	19.5	None	Caused slight excretion of creatine	75%. Decreased following glycine therapy	
Nevin ¹⁵	?	F	—	—	—	—	—	Phosphagen in resting muscles below normal and its breakdown during contraction sub-normal

*During control period.

†Milligrams of creatinine per kilogram per 24 hours.

‡Per cent of creatine retained.

§Values taken from graph of a representative case.

||Creatinine excretion of patient who excreted largest amounts of creatine.

TABLE II

EXCRETION OF CREATINE AND CREATININE BY THREE PATIENTS WITH DYSTROPHIA MYOTONICA

PATIENT	PERIOD	DAYS	GLYCINE FED (GM./DAY)	NUMBER OF URINE SPECIMENS	TOTAL CREATININE (GM./DAY)	PREFORMED CREATININE (GM./DAY)	CREATINE (GM./DAY)	CREATININE COEFFICIENT*
J. M.	4/21 to 4/28	8	None	3	1.02	0.91	0.13	14.5
	4/29 to 5/ 2	4	5.4	3	1.05	0.92	0.15	14.4
	5/ 3 to 5/ 5	3	10.8	2	1.11	0.93	0.22	14.6
	5/ 6 to 5/16	11	30.0	2	1.39	1.01	0.44	
	5/17 to 5/20	4	15.0	2	1.12	0.85	0.31	
	5/21 to 8/ 5	76	30.0	27	1.35	0.99	0.42	14.9
	8/ 6 to 12/16	133	30.0	0				
	12/17 to 12/19	3	30.0	3	1.21	0.90	0.36	
	12/20 to 1/11	23	None	0				
	1/12 to 1/15	4	None	2	1.14	0.98	0.19	15.2
N. P.	6/17 to 7/22	35	None	26	1.08	1.03	0.06	13.9
	7/23 to 7/27	5	15	4	1.07	1.02	0.06	
	7/28 to 8/ 5	9	30	7	1.15	1.08	0.07	14.0
	8/ 6 to 8/21	16	30	0				
	8/22 to 12/25	126	10-30	0				
	12/26 to 1/ 9	15	30	0				
	1/10 to 1/13	4	30	3	1.26	1.17	0.11	
	1/14 to 1/27	14	None	14	1.15	1.05	0.11	14.0
R. B.	1/19 to 2/ 1	14	None	5	1.09	1.03	0.07	22.4†
	2/ 2 to 2/25	23	10-30	6	1.15	1.10	0.05	
	2/26 to 3/23	26	15-30	2	1.28	1.23	0.05	
			(anterior pituitary extract also given§)					
	3/24 to 4/ 7	15	None	0				
	4/ 9 to 4/24	16	15-30 (testosterone propionate also given¶)	3	1.22	1.21	0.01	

*Milligrams of creatinine per kilogram per 24 hours.

†Based on weight of patient on Jan. 8.

‡Values the same in middle of period as at end of period.

§A total of 20 c.e. of anterior pituitary extract (Squibb) was administered in twelve injections (three times a week).

||Specimens collected at end of period.

¶A total of 135 mg. of oretan was administered in twelve injections.

tration. According to Milhorat and Wolff,¹³ the excretion of creatine is much less than in patients with progressive muscular dystrophy who show similar degrees of muscular disability. (4) Creatinine excretion is variably decreased.

EXPERIMENTAL

The excretion of creatine and creatinine was studied in one woman (J. M.) and two men (N. P. and R. B.) with dystrophia myotonica. J. M., female, aged 44 years, had severe myotonia in the hand grasps, and moderate atrophy of the forearms, hands, and lower extremities. She was still menstruating although somewhat irregularly. Her basal metabolic rate was minus 10. N. P., male, aged 52 years, had slight myotonia, marked atrophy of the forearms and hands, and

moderate atrophy of the lower extremities, but no evidence of testicular atrophy. His basal metabolic rate was minus 20. R. B., male, aged 27 years, had moderate myotonia, moderate atrophy of the forearms and hands and slight atrophy of the lower extremities. His testicles were small and questionably atrophic. His basal metabolic rate was minus 30. A more detailed description of these patients may be found in an earlier paper.²⁶

Determinations of creatine and creatinine in twenty-four-hour urines (7:00 A.M. to 7:00 A.M.) were made during a preglycine period, during glycine administration, and after discontinuing glycine. The amino acid was administered by mouth three times daily after meals in amounts as high as 30 Gm. per day. No attempt was made in any case to control the diet.

Preformed creatinine was determined by the Folin colorimetric method³ using a Duboseq colorimeter and matching through a piece of cobalt glass. Total creatinine was determined by the same method after the urine had been autoclaved with picric acid according to the method of Folin.⁵ Creatine was calculated by multiplying the difference in the above determinations by the factor 1.159.

Average values for the different experimental periods on these three patients are shown in Table II.

The woman patient, J. M., showed a creatinuria which was significantly higher than the average daily excretion of the normal women studied by Rose.²¹ In this patient creatine excretion increased definitely during glycine administration; the increase was prompt and the excretion remained high as long as glycine was fed, a period of eight months. The amount of creatine in the urine depended apparently on the amount of glycine fed, for it increased progressively as the dosage of glycine was raised and fell when the dosage of glycine was lowered. Three weeks after cessation of glycine administration the creatinuria had returned almost to the preglycine level.

Both of the men patients showed a slight initial creatinuria. The amounts of creatine excreted were approximately the same as those reported in patients with dystrophia myotonica by most other investigators, but these values are not as great as some of those reported for normal men by Taylor and Chew²⁵ and by Milhorat and Wolff.¹¹ In neither man was there any immediate effect on creatine excretion when glycine was fed; in patient R. B. there was no effect at any time. However, after glycine had been fed to patient N. P. for five and one-half months, the creatinuria had increased from the preglycine level of 0.06 Gm. per day to 0.11 Gm. per day, and it was still at this higher level two weeks after cessation of glycine feeding. Such a small increment in creatine excretion may have little significance, however, since Braestrup³ found that 14 Gm. of glycine daily produced a maximum excretion of 0.080 Gm., 0.094 Gm., and 0.100 Gm. per day in three normal men who had shown no initial creatinuria. On the other hand, Braestrup³ found that glycine produced no creatinuria in another normal man, and Milhorat and Wolff¹¹ reported that glycine feeding did not increase the creatine excretion of normal men who showed a preglycine creatinuria comparable to that of our two men patients. These reports were all concerned with relatively short periods of glycine feeding; no reports were found that showed

the effect on creatine excretion of administering glycine to normal men for such a prolonged period and in such large amounts as in our experiments.

Patient R. B., whose creatinuria was not augmented by glycine alone, had no greater excretion of creatine when anterior pituitary extract or testosterone propionate* was administered simultaneously with glycine. These results differ from those of Slauek²³ in a patient with dystrophia myotonica but are in accord with those of Milhorat and Wolff¹² in patients with myasthenia gravis. Slauek found that his patient, who showed no increase of creatine excretion in response to glycine alone, had a markedly increased creatinuria when testicular extract was administered simultaneously with glycine. Milhorat and Wolff reported that the administration of anterior pituitary extract to two patients with myasthenia gravis had no effect on creatine excretion. It is interesting to observe that Pizzolato and Beard¹⁷ have reported an increase in the creatine content of muscle and in the excretion of creatine when anterior pituitary extract is administered to normal rats, and also an increase in the creatine content of muscle when testicular extract is given.

Creatinine excretion in all three patients fluctuated considerably throughout the experiments and was not significantly affected by glycine administration. Part of the variation may be ascribed to changes in diet from day to day. The creatinine coefficient was within the normal range in two of the patients but was low in patient N. P., who showed the greatest degree of muscle atrophy.

SUMMARY AND CONCLUSIONS

1. The excretion of creatine and creatinine and the effect of glycine administration thereon were studied in three patients, one woman and two men, with dystrophia myotonica.

2. Creatinuria was present in all three patients during a preglycine period. The amounts of creatine excreted by the woman were greater than normal; the amounts excreted by the two men were small and probably within normal limits.

3. Glycine administration produced an increase in the amounts of creatine excreted by the woman and by one of the men. In the woman a definite increase occurred promptly; in the man the rather slight increase was slow to develop and of questionable significance. Neither the administration of glycine alone, of glycine and anterior pituitary extract, nor of glycine and testosterone propionate affected the excretion of creatine in the other man.

4. The excretion of creatinine was subnormal in the patient who showed the greatest degree of muscle atrophy but was normal in the other two patients. It was not significantly affected by glycine administration in any of the three patients.

5. Although the data obtained are insufficient for drawing definite conclusions, they seem to augment the evidence from previous reports that the slight to moderate creatinuria which may be observed in dystrophia myotonica is neither as constant nor as striking a finding as that observed in progressive muscular dystrophy, and is less strongly affected by administration of glycine than in the latter disease.

*The testosterone propionate (oretan) was furnished through the kindness of Dr. Max Gilbert of Shering Corporation.

REFERENCES

1. Adams, M., Power, M. H., and Boothby, W. M.: The Influence of Glycine on the Excretion of Creatine and Creatinine, *Am. J. Physiol.* 111: 596, 1935.
2. Burger, M.: Beiträge zum Kreatinstoffwechsel. II. Die Kreatin- und Kreatininausscheidung bei Störungen des Muskelstoffwechsels, *Ztschr. f. d. ges. exper. Med.* 9: 361, 1919.
3. Braestrup, P. W.: The Action of Glycine (Glycocol) on Muscular Dystrophy and Other Diseases, *Acta med. Scandinar.* 89: 231, 1935.
4. D'Antona, L.: Tentativi di terapia glicocollica della distrofia miotonica. Osservazioni sul ricambio creatinico, *Atti d. r. Accad. d. fisioerit. in Siena (Studi d. fac. med. senese)* 2: 647, 1934.
5. Folin, O.: On the Determination of Creatinine and Creatine in Urine, *J. Biol. Chem.* 17: 469, 1914.
6. Kolb, L. C., Harvey, A. M., and Whitehill, M. R.: A Clinical Study of Myotonia Atrophica and Myotonia Congenita With Special Reference to the Therapeutic Effect of Quinine, *Bull. Johns Hopkins Hosp.* 62: 188, 1938.
7. Kostakow, S., and Slauck, A.: Die Glykokollbehandlung der progressiven Muskeldystrophie, *Deutsche med. Wchnschr.* 59: 169, 1933.
8. Meyer, E. C.: Über Kreatin- und Kreatininausscheidung bei Krankheiten, *Deutsches Arch. f. klin. Med.* 134: 219, 1920.
9. Milhorat, A. T., and Wolff, H. G.: The Metabolism of Creatine in Muscle Disease, *J. Clin. Investigation* 13: 723, 1934.
10. Milhorat, A. T., and Wolff, H. G.: The Metabolism of Creatine in Muscle Disease, *Tr. Am. Neurol. A.* 60: 197, 1934.
11. Milhorat, A. T., and Wolff, H. G.: Studies in Diseases of Muscle. I. Metabolism of Creatine and Creatinine in Progressive Muscular Dystrophy, *Arch. Neurol. & Psychiat.* 38: 992, 1937.
12. Milhorat, A. T., and Wolff, H. G.: Studies in Diseases of Muscle. III. Metabolism of Creatine and Creatinine in Myasthenia Gravis, Including a Study of the Excretion of Nucleosides and Nucleotides, *Arch. Neurol. & Psychiat.* 39: 354, 1938.
13. Milhorat, A. T., and Wolff, H. G.: Studies in Diseases of Muscle. V. Metabolism of Creatine and Creatinine in Myotonia Congenita, Myotonia Atrophica, Amyotonia Congenita, Dystonia Musculorum Deformans and Paralysis Agitans, *Arch. Neurol. & Psychiat.* 40: 680, 1938.
14. Morgulis, S., and Young, A.: Metabolism in Myotonia Atrophica, *Arch. Int. Med.* 48: 569, 1931.
15. Nevin, S.: A Study of Muscle Chemistry in Myasthenia Gravis, Pseudohypertrophic Muscular Dystrophy and Myotonia, *Brain* 57: 239, 1934.
16. Pemberton, R.: A Metabolic Study of Myotonia Atrophica, *Am. J. M. Sc.* 141: 253, 1911.
17. Pizzolato, P., and Beard, H. H.: Creatine-Creatinine Metabolism and the Hormones, *Endocrinology* 24: 358, 1939.
18. Ravin, A., and Waring, J. J.: Studies in Dystrophia Myotonica. I. Hereditary Aspects, *Am. J. M. Sc.* 197: 593, 1939.
19. Ravin, A.: Studies in Dystrophia Myotonica. III. Experimental Studies in Myotonia, *Arch. Neurol. & Psychiat.* 43: 649, 1940.
20. Ravin, A., and Waring, J. J.: Studies in Dystrophia Myotonica. IV. Myotonia. Its Nature and Occurrence, *Ann. Int. Med.* 13: 1174, 1940.
21. Rose, M. S.: Creatinuria in Women, *J. Biol. Chem.* 32: 1, 1918.
22. Rymer, M. R., and Ravin, A.: Studies in Dystrophia Myotonica. VI. Results of Glucose Tolerance Tests, *J. Lab. & Clin. Med.* To be published.
23. Slauck, A.: Die therapeutische Beeinflussbarkeit der Dystrophia myotonica. Zugleich ein Beitrag zur Kenntnis vom intermediären Stoffwechsel des Muskels, *Verhandl. d. deutsch. Gesellsch. f. inn. Med., Kong.* 45: 175, 1933.
24. Stevenson, L.: Myotonia Atrophica, Cecil—Textbook of Medicine, Philadelphia and London, 1938, W. B. Saunders Co., p. 1375.
25. Taylor, F. H. L., and Chew, W. B.: Creatinuria in Adult Males, *Am. J. M. Sc.* 191: 236, 1936.
26. Waring, J. J., Ravin, A., and Walker, C. E.: Studies in Dystrophia Myotonica. II. Clinical Features and Treatment, *Arch. Int. Med.* 65: 763, 1940.

BIOCHEMICAL STUDIES OF THE BLOOD OF DOGS WITH N-PROPYL DISULFIDE ANEMIA*

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PREVIOUS studies by this laboratory of pernicious anemia¹ and the hemolytic and hypochromic anemias of childhood² have shown that chemical changes occur in the blood which are indicative of abnormal erythrocyte composition or structure. Further study and elucidation of the chemical structure of the normal erythrocyte has been made possible by the development of a method³ for the preparation of posthemolytic residue or stroma practically free of hemoglobin. Chemical studies of this material have revealed that the stroma of the normal erythrocyte is a protein-lipid complex of similar composition for various mammalian species.^{4, 5} Moreover, it has been demonstrated that the lipid distribution in the posthemolytic residue is similar to that in the intact erythrocyte.⁴

In view of the indications of abnormal erythrocyte structure in certain anemias, it seemed desirable to secure sufficient stroma for a complete chemical investigation of its character, together with hematologic and chemical observations on the intact erythrocyte. At present enough blood from human patients with anemias for preparation of sufficient stroma is practically impossible to secure. It is possible, however, to develop an experimental anemia in animals: a procedure which offers the advantages of more rigid control, but has disadvantages in that the particular types of anemia occurring in human beings which present the most striking changes in erythrocyte structure^{1, 2} are not amenable to experimental development. Simple hypochromic anemia (iron deficiency), which results in impaired hemoglobin formation, does not appear to affect the chemical design of the erythrocyte.² Hemolytic anemias, which are accompanied by definite alterations in the hematologic pattern of the blood, are produced in animals by certain drugs causing red blood cell destruction. Such an anemia offers an excellent opportunity for correlated hematologic, chemical, and stroma studies which are important in view of the marked abnormalities of erythrocyte composition found in the congenital hemolytic anemias of childhood.²

Sebrell⁶ originally observed that onions would produce a severe anemia in dogs. Grulhitz^{7, 8} confirmed this and showed further that the action resided in

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the onion oil, probably due to the allyl propyl disulfide, the main constituent of onion oil. In a study of different disulfide compounds Grulhitz⁸ demonstrated that N-propyl disulfide produced a severe anemia in dogs and that any desired degree of anemia can be maintained for a long period of time, depending on the regular administration of definite amounts of the disulfide, a significant factor in studying the influence of anemia on erythrocyte structure.

Grulhitz⁸ has described the changes in the hematologic picture induced by this type of anemia, but thus far no chemical observations have been reported. Furthermore, the possibility of using N-propyl disulfide to control polycythemia in human beings⁹ points to the necessity of studying not only the hematologic and chemical changes occurring in the blood but also the pathologic changes in the tissues as well. This report includes, therefore, detailed chemical and hematologic examinations of the blood and pathologic observations of certain tissues, in addition to the effect of anemia on the composition of erythrocyte stroma.

EXPERIMENTAL

Two originally healthy dogs were maintained in a moderate-to-severe degree of anemia for a period of over three months by the administration of N-propyl disulfide ($\text{CH}_3\text{CH}_2\text{CH}_2\text{S-SCH}_2\text{CH}_2\text{CH}_3$).⁶ Previous to beginning the study, the animals were immunized with *B. bronchisepticus* vaccine. The dogs were fed a complete, nutritive diet and given the disulfide in gelatin capsules at intervals and dosages, as indicated in Chart 1. Hematologic examinations consisting of hemoglobin determinations, red blood cell and white blood cell counts were made at intervals during the course of the experiment. These values, plotted in Chart 1, show the degree and maintenance of the anemias as the study progressed. Samples of blood (60 c.c.) for chemical analysis were taken at the beginning of the experiment before disulfide was administered, frequently in the first month during the period of adjustment, and at the end of the study, as indicated in Chart 1. Determinations of red blood cell count, hemoglobin content, hematocrit value, red blood cell diameter, specific gravity, and water content were made, from these, corpuscular volume, weight, thickness, area, hemoglobin, and water were calculated. The chemical determinations on the blood included the following: serum calcium and inorganic phosphorus, total protein of the plasma; sodium, potassium, and chloride content of serum and cells and the complete lipid distribution of the plasma and erythrocytes. The methods used were the same as those in previous studies reported from this laboratory.^{10, 11}

Just preceding completion of the experiment, x-rays[†] of the skull, spine, and leg bones of both dogs were made. When the experiment was terminated, the dogs were exsanguinated under light ether anesthesia and the blood was used for the preparation of erythrocyte stroma.³ Tissues were preserved for histopathologic and chemical study.

⁶This study was made possible through the courtesy and facilities of Parke, Davis & Co., Detroit. E. A. Sharp, M.D., medical director, suggested the use of N-propyl disulfide to produce the anemia and gave helpful suggestions during the course of the investigation. L. T. Clark, Ph.D., director of the biological laboratories, made the animals available and the investigation possible. O. M. Grulhitz, M.D., pathologist, prepared the anemic animals, made the hematologic examinations on the blood, took the blood and tissue samples, and gave helpful advice in the preparation of the manuscript.

[†]The x-ray films were taken and read by Lawrence Reynolds, M.D., roentgenologist, Harper Hospital, Detroit.

TABLE I
HEMATOLOGIC OBSERVATIONS ON DOGS WITH DISULFIDE ANEMIA

DOG	HEMATO- CRIT VALUES (%)	RED BLOOD CELLS		HEMOGLOBIN		CORPUSCULAR MEASUREMENTS					WATER PER CELL (μ g)
		WHOLE BLOOD (ML- LIONS/ C.M.M.)	PER GRAM CELLS ($\times 10^{10}$)	WHOLE BLOOD (GM./100 C.C.)	PER CELL (μ g)	VOLUME (C. μ)	WEIGHT (μ g)	DIAMETER (μ)	THICK- NESS (μ)	AREA (SQ. μ)	
Normal.* Average Range	51 48-53	6.4 5.8-7.3	1.15 1.11-1.26	13.8 12.5-15.0	22 19-26	80 73-83	87 80-90	6.8 6.6-6.9	2.3 2.2-2.4	120 118-123	55 52-61
146: Pre-experimental After induction of anemia: Within first month: Average† Range After 3 months	43 25 18-33 30	6.5 3.4 2.0-4.6 3.3	1.38 1.19 0.99-1.31 1.00	11.7 6.3 3.4-8.8 8.2	18 19 13-23 25	66 77 71-93 93	72 86 76-101 100	- 7.5 7.4-7.5 7.4	- 1.8 1.6-2.1 2.2	- 129 126-138 137	- 57 53-67 68
148: Pre-experimental After induction of anemia: Within first month: Average† Range After 3 months	44 23 18-29 32	7.1 3.0 1.7-4.2 3.5	- 1.20 0.86-1.33 1.03	12.9 5.3 3.9-7.3 7.8	- 18 14-27 22	- 80 70-108 90	- 87 75-116 98	- 7.4 7.3-7.6 7.5	- 1.9 1.6-2.4 2.0	- 130 123-148 135	- 60 52-76 66

*Five determinations on 3 healthy dogs.

†Average for 1 samples.

‡Average for 5 samples.

TABLE II
DISTRIBUTION OF WATER, PROTEIN, AND MINERALS IN BLOOD OF DOGS WITH DISULFIDE ANEMIA

DOG	RED BLOOD COUNT (MILLIONS/C.M.M.)	SERUM INORGANIC PHOSPHORUS (MG./100 C.C.)	SERUM CALCIUM (MG./100 C.C.)	PLASMA PROTEIN (GM./100 C.C.)	SERUM				ERYTHROCYTES				CORPUSCULE		
					SODIUM (MEQ./LITER)	POTAS- SIUM (MEQ./ LITER)	CHLO- RIDE (MEQ./ LITER)	WATER (GM./ LITER)	SODIUM (MEQ./ LITER)	POTAS- SIUM (MEQ./ LITER)	CHLO- RIDE (MEQ./ LITER)	WATER (GM./ LITER)	SODIUM (MEQ. X 10-13)	POTAS- SIUM	CHLO- RIDE
Normal; Average Range	6.4 5.8-7.3	4.2 3.0-5.3	12 11-13	6.2 5.9-6.6	148 140-152	8 5-11	110 107-112	944 942-946	118 114-128	7 4-13	52 41-58	717 701-732	94 91-96	6 3-11	41 30-46
146: Pre-experimental After induction of anemia: Within first month; Average Range After 3 months	6.5 3.4 2.0-4.6 3.2	4.8 5.4 3.1-5.9 4.3	12 12 11-12 12	6.7 6.8 6.2-7.4 6.2	143 148 147-149 150	- 15 6-17 6	100 116 112-120 96	939 938 935-940 944	109 100 78-121 106	- 7 3-12 21	52 58 47-78 25	724 732 718-738 730	72 77 73-88 99	- 5 2-8 20	35 46 36-57 23
148: Pre-experimental After induction of anemia: Within first month; Average Range After 3 months	7.1 3.0 1.7-4.2 3.3	3.9 5.1 4.4-5.5 4.7	12 11 10-12 -	7.2 6.7 6.3-7.6 6.5	146 145 138-150 146	- 8 5-10 5	105 112 104-113 93	- 938 932-942 937	110 120 103-165 115	- 25 6-37 19	53 66 56-82 28	- 741 735-749 734	88 94 75-115 104	- 18 6-26 17	42 51 45-60 26

*Four determinations on 3 healthy dogs.

†Average of 4 samples.

‡Average of 5 samples.

RESULTS

The clinical blood picture during the course of the anemia is given in Chart 1 and demonstrates the marked anemia producing powers of N-propyl disulfide and, furthermore, shows the closely parallel responses of the two dogs to the drug. Table I presents the hematologic observations on the blood samples taken for chemical analysis. Before administration of the disulfide the two dogs had blood counts of 6.5 and 7.1 millions per cubic millimeter, respectively. During the first month of the study these values ranged between 1.7 and 4.6 millions, after which the level approximated 3.5 millions for both animals until the termination

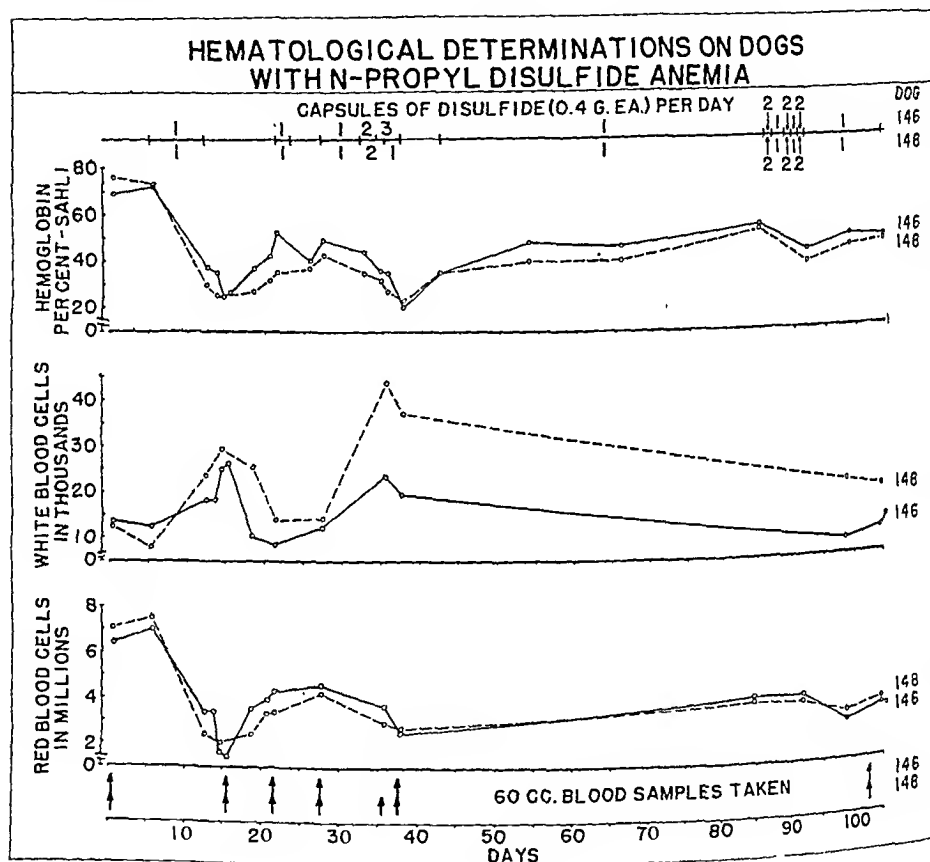


Chart 1.

of the experiment. Specific gravity, water, and protein contents of the plasma showed no change as a result of the anemia (Table II), which indicates that the blood concentration was unaffected. All corpuscular measurements disclosed a larger and heavier cell in the anemic state. The cell volume was about 80 c. μ in the animals before anemia was induced, but with the progress of the anemic condition the cell volume increased to 90 c. μ or over. Likewise, the corpuscular weight was 90 micromicrograms or less in the nonanemic bloods, but increased to a weight of 98 micromicrograms or more in the anemic condition. Similarly, the cell diameter and the calculated cell area were increased with the anemia, whereas the computed number of cells per gram of cells was decreased. Although part of

the increased weight may have been due to a larger quota of hemoglobin per cell, it is accounted for chiefly by an augmented water content. Whereas the corpuscular water of the normal dog erythrocytes averaged 55 micromicrograms, it rose as high as 76 micromicrograms during the anemia, changes accompanied are shown by a corresponding decrease in specific gravity. These alterations indicate that the cells underwent osmotic swelling, which is in accord with their increased fragility in hypotonic sodium chloride solutions. Hemolysis was evident in 0.62 per cent sodium chloride (the highest concentration used) in comparison with normal values of 0.50 to 0.54 per cent given by Haden.¹²

The sodium, potassium, and chloride distribution of the blood serum and cells, together with the serum calcium and inorganic phosphorus values, are given in Table II. The plasma mineral constituents, except the chloride, appear to be unaffected by the induction of the anemia. The chloride content of both the serum and erythrocytes was consistently lower after three months of anemia. Serum chloride was above 100 meq. per liter in the animals without anemia, while at the end of the study it was 96 meq. or less. Similarly, the chloride content of the erythrocytes was reduced markedly, from above 50 meq. in the animals when nonanemic to below 30 meq. after three months' duration of the anemia.

TABLE III

DISTRIBUTION OF MINERALS IN WATER OF PLASMA AND ERYTHROCYTES
(Meq. per liter of water)

DOG	PLASMA				ERYTHROCYTES			
	SODIUM	POTASSIUM	TOTAL BASE	CHLORIDE	SODIUM	POTASSIUM	TOTAL BASE	CHLORIDE
Normal:*	157	9	163	117	164	10	174	73
146:								
Pre-experimental	152	-	-	116	151	-	-	72
Within first month†	157	16	173	124	137	10	147	78
After 3 months	159	6	165	102	144	29	175	34
148:								
Within first month‡	153	9	164	112	162	34	196	80
After 3 months	155	5	160	100	156	27	183	38

*Four determinations on 3 healthy dogs.

†Average of 4 samples.

‡Average of 5 samples.

The advantages of expressing the erythrocyte composition in terms of an individual corpuscle rather than on the unit weight basis has been demonstrated in the anemias.^{1, 2} In this study, calculation of the corpuscular mineral concentration emphasizes further the lowered chloride content of the erythrocyte at the termination of the experiment (Table II). In addition, there is evidence of an elevated base (sodium and potassium) content per cell which is not particularly apparent from the values expressed in milliequivalents per liter of erythrocytes. Before anemia the dogs had 72 and 88 $\times 10^{-13}$ meq. of sodium per cell, whereas after three months in the anemic state, there were 99 and 104 $\times 10^{-13}$ meq. of sodium, respectively, in comparison with normal values of 91 to 96 $\times 10^{-13}$ meq. Likewise, potassium was elevated after duration of the anemia to 17 and 20 $\times 10^{-13}$ meq. of potassium in contrast to a normal value of 3 to 6 $\times 10^{-13}$ meq. per

DOG	RED BLOOD COUNT (MIL- LIONS/ C.MM.)	PLASMA (MG./100 C.C.)							ERYTHROCYTES	
		TOTAL LIPID	NEU- TRAL FAT	FREE CHO- LES- TEROL	CHOLE- STEROL ESTERS	PHOSPHOLIPID			TOTAL LIPID	NEU- TRAL FAT
						TOTAL	CEPHA- LIN	CHO- LINE§		
Normal:*										
Average	6.4	632	137	31	156	308	82	226	615	99
Range	5.8-7.3	583-680	130-148	19-43	144-174	268-357	44-153	204-245	448-699	0.14
146:										
Pre-experi- mental	6.5	566	69	37	174	286	69	217	727	183
After induc- tion of anemia:										
Within first month:										
Average†	3.4	565	150	41	154	221	49	172	1035	283
Range	2.0-4.6	488-674	82-203	38-43	132-194	171-277	21-72	138-205	487-1408	2147
After 3 months	3.2	412	173	33	70	136	0	136	793	159
148:										
Pre-experi- mental	7.1	446	22	35	119	270	108	162	934	374
After induc- tion of anemia:										
Within first month:										
Average†	3.0	576	143	40	148	244	60	175	931	192
Range	1.7-4.2	459-739	70-183	31-59	115-211	143-338	40-104	103-260	682-1407	043
After 3 months	3.5	419	161	28	77	153	0	153	881	313

*Five determinations on 3 healthy dogs.

†Average of 4 samples.

‡Average of 5 samples.

§Lecithin plus sphingomyelin.

**Represents the concentration of an average single red blood cell.

cell. The elevated corpuscular base, together with the lowered chloride, indicates an excess cation content or the presence of increased amounts of an undetermined anion, alterations which are similar to those noted in various types of human anemias.^{1, 2, 13}

The anemia was accompanied by changes in the erythrocytes indicative of osmotic swelling. Calculation of the mineral composition on the basis of milliequivalents per liter of cellular and plasma water (Table III) bring out the fact that normally, as well as in the anemia, the sodium is almost evenly distributed between the water of plasma and cells. Normally, the potassium is likewise equally partitioned, but as the anemia progresses it becomes more concentrated in the cells. On the other hand, although the chloride is lowered in the water of both plasma and cells, it is more markedly diminished in the cells, which is in agreement with the swelling of the cell and its increased fragility.

The lipid composition of the plasma and erythrocytes (mg. per 100 c.c.), together with the calculated corpuscular concentration (mg. $\times 10^{-12}$ per cell), are given in Table IV. The total lipid content of the plasma of the dogs before

WITH DISULFIDE ANEMIA

(MG./100 C.C.)				CORPUSCLE (MG. $\times 10^{-12}$)**						
CHOLESTEROL ESTERS	PHOSPHOLIPID			TOTAL LIPID	NEUTRAL FAT	FREE CHOLESTEROL	CHOLESTEROL ESTERS	PHOSPHOLIPID		
	TOTAL	CEPHALIN	CHOLINE					TOTAL	CEPHALIN	CHOLINE
15	369	187	182	492	81	104	12	295	149	146
18	0-35	271-467	150-240	327-574	0-156	85-118	0-26	198-386	123-198	72-209
26	442	274	168	483	122	50	17	293	182	112
0	593	352	241	820	221	123	0	476	272	203
11	0-6	293-916	293-444	353-1311	17-335	105-154	0-0	212-853	212-324	0-529
130	351	112	239	738	140	151	121	326	104	222
50	372	190	182	747	299	106	45	298	152	146
40	542	255	287	726	140	123	30	432		240
29	0-98	350-593	0-493	504-979	0-362	75-159	0-68	264-642	183	0-642
17	390	74	816	794	282	145	15	352	0-364	285
								67		

anemia ranged between 446 and 680 mg. At the end of the study the experimental animals had a plasma lipid content of 412 and 419 mg. Of the various components of the total lipid, free cholesterol appears to have been unaffected by the induction of the anemia; on the other hand, neutral fat was increased and cholesterol esters and phospholipid were decreased. Although dogs 146 and 148 had a lower plasma neutral fat, 69 and 22 mg., respectively, before induction of the anemia as compared to an average of 137 mg. for the animals that received no drug, they had concentrations of 173 and 161 mg. at the termination of the experiment. The cholesterol ester concentration of the plasma ranged between 119 and 174 mg. in the nonanemic animals. After three months of N-propyl disulfide the anemic dogs (146 and 148) had a plasma content of 70 and 77 mg. of cholesterol esters, respectively. Similarly, the plasma phospholipids ranged between 270 and 357 mg. in the animals when healthy, and fell after three months of anemia to 136 mg. in dog 146 and 153 mg. in dog 148. Separation of the phospholipid fraction into cephalin and choline phospholipid (lecithin plus sphingomyelin) showed that, whereas both follow a trend similar to the total phospholipid, the cephalin appears to be affected to a greater and more consistent degree. In fact, no cephalin could be detected in the plasma of the dogs after being kept anemic for three months with disulfide. These parallel changes in the plasma

lipids of the two dogs—lowered phospholipid and cholesterol esters and increased neutral fat—are typical of the changes found in the plasma lipids of different types of anemias in man and animals.^{1, 2, 14}

A study of erythrocyte lipids (on the basis of milligrams per 100 c.c. of cells) indicates that although the total lipid concentrations in the two animals varied widely during the first month of the anemia, the values at the end of the experiment appear comparable to those obtained before N-propyl disulfide was given.⁴ The neutral fat concentration shows the widest variation of any of the lipid components, but without relation to the anemia. Free cholesterol seems to have been increased, and the total phospholipid appears to have undergone no consistent change as the result of the anemia.

Due to the changing size and weight of the red blood cells in anemia, however, determinations on the unit weight basis may obscure what is actually occurring in the individual corpuscle. Calculation of the composition of the individual cell, therefore, gives a truer picture of the chemical changes in structure, well illustrated by the present study. Both dogs exhibited an increased corpuscular concentration of total lipid not evident from the data on total lipid per 100 c.c. of erythrocytes. Neutral fat data show no change attributable to the anemia. The increase in free cholesterol is brought out more strikingly in the corpuscular concentration. The animals which received no disulfide had a range of 85 to 115 mg. $\times 10^{-12}$ per cell, and the experimental dogs had values of 50 and 106 $\times 10^{-12}$ before administration of the drug. At the end of the three months of anemia there was an increase to 151 and 145 mg. $\times 10^{-12}$ per cell, respectively.

The rise in cholesterol ester content of the corpuscle, noted in previous studies of anemia,^{1, 2} was observed in dog 146; the cholesterol esters increased from 17 $\times 10^{-12}$ mg. per cell to 121 $\times 10^{-12}$ mg. at the end of the experiment. The other experimental animal (dog 148) showed little or no change in the corpuscular content of cholesterol esters.

The concentration of the corpuscular phospholipid was increased in both dogs as a result of the anemia, in contrast to the observations per liter of cells. The average concentration of phospholipid per single cell was 295 $\times 10^{-12}$ mg. for the untreated animals and 293 and 298 $\times 10^{-12}$ mg., respectively, for dogs 146 and 148 before administration of the disulfide.

During the first month of anemia the total phospholipid was extremely elevated at times (853 and 642 $\times 10^{-12}$ mg.), producing average values of 476 and 432 $\times 10^{-12}$ mg., larger amounts than after three months in the anemic state when the values were 326 and 252 $\times 10^{-12}$ mg. per cell, respectively, for dogs 146 and 148. When the total phospholipid is separated into its individual components, salient differences are revealed. Cephalin was significantly elevated at times within the first month, whereas the choline phospholipids (lecithin plus sphingomyelin) reached both minimum and maximum values. There may be a close relationship between the presence of immature red blood cells, the elevated total phospholipid, and the individual phospholipid partition at the initiation of the anemia. Rapid erythrocyte destruction by the drug is followed by an outpouring of reticulocytes⁵ which in this study occurred during the first month. With longer duration of the anemia the marked decrease in corpuscular cephalin and

elevated choline phospholipid indicate a definite change in the structure of the red blood cell. These results support recent views that hemolytic agents injure directly the structure of the mature erythrocyte, making it more susceptible to normal hemolytic processes in the body.¹⁵

TABLE V
LIPID DISTRIBUTION OF BRAINS

DOG	TOTAL LIPID	TOTAL PHOS- PHO- LIPID	FREE CHO- LES- TEROL	CHO- LES- TEROL ES- TERS	NEU- TRAL FAT	CERE- BRO- SIDES	CEPH- ALIN	LECI- THIN	SPHIN- GO- MYE- LIN	WATER
<i>Per Cent Wet Weight</i>										
Normal:										
93	10.3	5.0	1.9	0.0	2.2	1.0	3.0	0.5	1.4	75.4
94	8.8	4.6	1.8	0.0	1.4	0.9	2.7	1.1	0.8	77.1
Anemic:										
145	9.1	4.6	1.8	0.4	0.6	1.6	3.0	0.7	0.8	77.1
148	11.0	5.0	2.3	0.1	1.2	2.2	2.8	0.9	1.2	74.8
<i>Per Cent Dry Weight</i>										
Normal:										
93	41.4	20.1	7.6	0.0	8.8	4.0	12.1	2.0	5.6	
94	38.2	20.0	7.8	0.0	6.1	3.9	11.7	4.8	3.5	
Anemic:										
145	39.8	20.1	7.9	1.8	2.6	7.0	13.1	3.1	3.5	
148	43.9	19.9	9.2	0.4	4.8	8.8	11.2	3.6	4.8	

In view of the neurologic manifestations which frequently accompany anemia, detailed lipid analyses and histopathologic studies* of the brains were made (Table V). No significant differences are evident in any of the lipid constituents except cerebrosides, which was slightly higher, and neutral fat, which was lower in the brains of the anemic dogs. Cerebroside values† for the anemic dogs were 1.6 and 2.2 per cent (wet weight) as compared to 1.0 and 0.9 per cent for normal dogs; neutral fat values were 0.6 and 1.2 per cent as compared to 2.2 and 1.4 per cent, respectively. The brains contained (on the wet weight basis) 5 per cent total phospholipid, 2 per cent free cholesterol, and negligible amounts of cholesterol esters. Cephalin constituted the major portion of phospholipid (60 per cent of the total), with the remaining 40 per cent about equally partitioned as lecithin and sphingomyelin.‡

Histopathologic examinations§ of the liver, spleen, and bone marrow of each animal showed that conspicuous changes had occurred with the anemia. The livers were found to be congested and contained pigment deposits. There was no evidence of an infectious process. The spleens contained a large amount of light brown pigment and the sinusoids were distended with red blood cells to such a degree that most of the normal splenic structure was indistinct. The appearance

*Each brain was divided into six separate sections, which were analyzed separately and then totaled for values on the entire brain. Specimens of the separate sections of each brain were taken for histopathologic examination by Gabriel Steiner, M.D., research professor of neurology and neuropathology, Wayne University College of Medicine, Detroit, and the results will be related with the chemical analyses in a later report.

†Cerebrosides were determined by a modification of the Kimmelstiel-Kirk¹⁶ method.

‡Micro methods for lecithin, cephalin, and sphingomyelin developed in this laboratory are described in a separate publication.¹⁷

§The bone marrow smears were observed by Pearl Lee, M.D., hematologist, and the liver and spleen by Martha Madsen, M.D., pathologist, Children's Hospital of Michigan, Detroit.

was similar to the active stage of hemolytic icterus. Although x-ray examination* of the bones of the animals failed to reveal any noticeable changes, the bone marrow smears showed an increase in nucleated red blood cells and neutrophilic myelocytes, a picture of a hyperactive bone marrow. These results confirm those of Gruhzit⁹ on the hemolytic nature of the disulfide anemia.

A review of the physical and chemical changes which occurred in the erythrocytes as the result of the disulfide anemia discloses further similarities to the abnormal red blood cells in active hemolytic icterus²; the enlargement of the cell accompanied by increases in fragility, water, and total base content is particularly analogous.

TABLE VI

LIPID AND PROTEIN COMPOSITION OF POSTHEMOLYTIC RESIDUE OF NORMAL AND ANEMIC DOG BLOOD

(Per cent of dry weight)

	NORMAL	AFTER 3 MO. OF ANEMIA
Dogs	93 and 94	146 and 148
Total lipid	16.9	11.6
Neutral fat	2.8	0.9
Free cholesterol	6.2	3.9
Cholesterol esters	0	1.7
Phospholipid	7.9	5.1
Cephalin	2.9	1.9
Lecithin	4.2	2.2
Sphingomyelin	0.8	1.0
Protein (N \times 6.25)*	57.1	70.5
Amino acid nitrogen:†		
Histidine	5.1	5.1
Arginine	11.5	9.9
Lysine	6.6	5.9
Tyrosine	2.1	2.1
Tryptophane	1.4	1.3

*Protein calculated on basis of total stroma.

†Per cent of total nitrogen of lipid-free stroma.

A more direct approach to the chemical changes which occur in the erythrocyte structure can be made by a study of the composition of the posthemolytic residue or stroma of the red blood cell.³⁻⁵ In order to secure a sufficient amount of this material for detailed lipid and protein analysis, it was necessary to combine all the blood that could be drawn from the two dogs with N-propyl disulfide anemia at the end of the study. The lipid and protein analyses of the stroma from the combined blood of the anemic animals (146 and 148) are presented in Table VI, together with similar analyses on the stroma obtained from the combined blood of two untreated animals.

The stroma isolated from the cells in anemia had a total lipid content of 11.6 per cent as compared to 16.9 per cent for that of the normal dog stroma. Likewise, the neutral fat (which includes cerebrosides) and free cholesterol fractions were lower in the stroma from the anemic dogs. Noticeable, however, is the appearance of cholesterol esters. In the normal dog stroma no cholesterol esters could be detected. In the anemic stroma there was found to be 1.7 per cent cholesterol esters. Both cephalin and lecithin components of the phospholipid were lower, paralleling the lower phospholipid content, whereas the remaining

*See footnote † page 997.

phospholipid constituent, sphingomyelin, was approximately the same in the stroma from the normal and anemic dogs. The diminished cephalin content of the stroma is in accordance with that observed for the erythrocyte as a whole, but the decreased lecithin indicates that not all of this phospholipid within the anemic cell is combined with the stroma. The decreases in total phospholipid and free cholesterol, together with the appearance of cholesterol esters, are indicative of degenerative and retrogressive tissue changes¹ and imply a defective or impaired structure of the erythrocyte in the anemic state.

The total protein content, in contrast to the lipid, was lower in the normal dog stroma (57.1 per cent) as compared to the anemic stroma (70.5 per cent). Determination of the stroma content of histidine, arginine, lysine, tyrosine, and tryptophane, showed that on the basis of these amino acids, which account for 24 to 27 per cent of the total protein nitrogen, the normal dog stromal protein is similar to the anemic stromal protein and to that from the erythrocytes of other mammalian species⁵. It appears, therefore, that the disulfide anemia has no qualitative effect on the erythrocyte stroma protein. In view of the acidic nature of this type of protein⁵ an increased proportion in the stroma, however, may be related to the elevated base content of the erythrocyte in the anemia.

SUMMARY

Correlated investigations of dogs with an experimental hemolytic anemia induced by N-propyl disulfide have included determination of hematologic, physical, and chemical characteristics of the blood; the chemical composition of the erythrocyte stroma; roentgenologic examinations of the bones and histopathologic observations of tissues.

As a result of the anemia, chloride, phospholipid and cholesterol esters were lowered in the serum, or plasma, and the neutral fat content was increased. The plasma lipid changes were similar to those observed in the hemolytic anemias of childhood and pernicious anemia and appear to be typical of all anemias.

In the N-propyl disulfide anemia the erythrocyte was larger and heavier, as shown by increased volume, weight, and diameter of the cells; an increase in water content accompanied by a lower specific gravity attended by greater fragility indicate that the anemia caused osmotic swelling of the red blood cells.

Elevated corpuscular base and lowered chloride contents, previously noted in anemias of man, point to an excess cation content or an increased amount of an undetermined anion in the erythrocyte of an anemic subject.

Marked alteration in the cellular structure of the erythrocyte in anemia is indicated by the changes in the lipid fractions; outstanding alteration was a striking decrease in cephalin content.

The chemical composition of the erythrocyte stroma confirms the altered red blood cell structure in anemia. The stroma, a complex of lipid and protein, was changed both quantitatively and qualitatively in its lipid content; the protein fraction of the stroma in anemia was similar in composition to normal stroma but larger amounts were present. It is significant, in view of the acidic nature of the stroma protein, that the red blood cells in anemia contained greater amounts of basic minerals.

REFERENCES

1. Williams, H. H., Erickson, B. N., Bernstein, S. S., Hummel, F. C., and Macy, I. G.: The Lipid and Mineral Distribution of the Serum and Erythrocytes in Pernicious Anemia: Before and After Therapy, *J. Biol. Chem.* 118: 599, 1937.
2. Erickson, B. N., Williams, H. H., Hummel, F. C., Lee, P., and Macy, I. G.: The Lipid and Mineral Distribution of the Serum and Erythrocytes in the Hemolytic and Hypochromic Anemias of Childhood, *J. Biol. Chem.* 118: 569, 1937.
3. Bernstein, S. S., Jones, R. L., Erickson, B. N., Williams, H. H., Avrin, I., and Macy, I. G.: A Method for the Preparation of Posthemolytic Residue or Stroma of Erythrocytes, *J. Biol. Chem.* 122: 507, 1938.
4. Erickson, B. N., Williams, H. H., Bernstein, S. S., Avrin, I., Jones, R. L., and Macy, I. G.: The Lipid Distribution of Posthemolytic Residue or Stroma of Erythrocytes, *J. Biol. Chem.* 122: 515, 1938.
5. Beach, E. F., Erickson, B. N., Bernstein, S. S., Williams, H. H., and Macy, I. G.: The Amino Acid Composition of Erythrocyte Posthemolytic Residue of Five Mammalian Species, *J. Biol. Chem.* 128: 339, 1939.
6. Sebrell, W. H.: An Anemia of Dogs Produced by Feeding Onions, *U. S. Pub. Health Rep.* 45: 1175, 1930.
7. Grulhitz, O. M.: I. Anemia of Dogs Produced by Feeding of the Whole Onions and of Onion Fractions, *Am. J. M. Sc.* 181: 812, 1931.
8. Grulhitz, O. M.: II. Anemia in Dogs Produced by Feeding Disulphide Compounds, *Am. J. M. Sc.* 181: 815, 1931.
9. Grulhitz, O. M.: Sulphide Anemia: Nonspecific Action of Anti-anemic Substances, *Arch. Path.* 14: 277, 1932.
10. Erickson, B. N., Williams, H. H., Hummel, F. C., and Macy, I. G.: The Lipid and Mineral Distribution in the Serum and Erythrocytes of Normal Children, *J. Biol. Chem.* 118: 15, 1937.
11. Williams, H. H., Erickson, B. N., Avrin, I., Bernstein, S. S., and Macy, I. G.: Determination of Cephalin in Phospholipids by the Estimation of Choline, *J. Biol. Chem.* 123: 111, 1938.
12. Haden, R. L.: The Mechanism of the Increased Fragility of the Erythrocytes in Congenital Hemolytic Jaundice, *Am. J. M. Sc.* 188: 441, 1934.
13. Maizels, M.: The Anion and Cation Contents of Normal and Anaemic Bloods, *Biochem. J.* 30: 821, 1936.
14. Peters, J. P., and Van Slyke, D. D.: Quantitative Clinical Chemistry, Vol. 1, Interpretations, Baltimore, 1931, Williams & Wilkins Co., p. 245.
15. Dameshek, W., and Schwartz, S. O.: Hemolysis as the Cause of Clinical and Experimental Hemolytic Anemias With Particular Reference to the Nature of Spherocytosis and Increased Fragility, *Am. J. M. Sc.* 196: 769, 1938.
16. Kirk, E.: A Study of Kimmelstiel's Procedure for Titrimetric Cerebroside Determination. With Description of an Improved Technique, *J. Biol. Chem.* 123: 613, 1938.
17. Erickson, B. N., Souders, H. J., Shepherd, M. L., Teague, D. M., and Williams, H. H.: Relation Between Cerebroside and Neutral Fat Contents of Blood Plasma, Erythrocytes and Stroma, *Proc. Soc. Exper. Biol. & Med.* 45: 153, 1940.
18. Erickson, B. N., Avrin, I., Teague, D. M., and Williams, H. H.: Micromethods for the Determination of Sphingomyelin and Choline. Applications for the Estimation of the Phospholipid Partition (Sphingomyelin, Lecithin, and Cephalin) in Blood and Tissues, *J. Biol. Chem.* 135: 671, 1940.

THE INFLUENCE OF THE PAROTID GLAND ON BLOOD SUGAR*

A PRELIMINARY REPORT

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THE existence of a carbohydrate-regulating hormone, secreted by the parotid gland, has been postulated by several foreign observers. Hiki¹ and his collaborators obtained parotid extracts, which, when injected into dogs, caused marked and rapid hyperglycemia. Takaes,² on the other hand, reported a hypoglycemia in animals following the injection of ether and alcohol extracts of this gland. Rosenfeld³ maintained that the hypoglycemia following the ligation of the parotid ducts is due to absorption of amylase or ptyalin, rather than to an internal secretion of an insulinlike substance. Thus biochemical studies have given rise to three differing conclusions concerning the action of parotid extracts.

Cahane and Cahane⁴ in 1924 extirpated the parotid glands of two dogs. They reported subsequent hypertrophy and hyperplasia of the islands of Langerhans. Annaps⁵ reported cure of hyperglycemia of depancreatized dogs following parotidectomy. Ferretti,⁶ on the other hand, stated that hypertrophy of the parotid glands is a compensatory mechanism; i.e., the parotids take on the function of the pancreas in the manufacture of insulin.

Injections of parotid extracts and surgical procedures on the parotid gland have had definite effects on blood sugars. These effects and the interpretations thereof have not been uniform.

The present study was undertaken, therefore, in order to study blood sugar levels and tissue changes in completely deparotidectomized animals.

EXPERIMENTAL PROCEDURES

Eight young male albino rats, weighing from 223 to 305 Gm., were used. A daily record of food consumption and weights was kept. Daily blood specimens were drawn at approximately the same time of day for a period of one week. The animals were not fasted prior to the drawing of blood, since there is no indication that greater precision in the estimation of blood sugars in rats could be secured by periods of inanition.⁷ Four rats were chosen at random for the operative procedure and four rats were used as controls. Total bilateral parotidectomies were performed on rats Nos. 3 and 5; one-sixth of the right parotid gland of rat No. 7 was left in situ. In rat No. 1 both glands were removed except for one-eighth of the right gland around which several ties were tightly placed. The modified microcolorimetric method of Benedict⁸ was used for the sugar determinations.

*From the Laboratories of the Delamar Institute of Public Health, Columbia University.
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RESULTS

Rat No. 3 showed a marked and significant decrease in the blood sugar from a preoperative mean of 110 mg. \pm 13 mg. per 100 c.c. of blood to 22 mg. per 100 c.c. of blood (Table I). The blood sugar remained low for three days. On the fourth day the reading returned to the preoperative level.

TABLE I
BLOOD SUGAR READINGS*

CONTROL ANIMALS				
ANIMAL NO.	PREOPERATIVE PERIOD		POSTOPERATIVE PERIOD	
	MEAN	STAND. DEV.	MEAN	STAND. DEV.
4	125	\pm 10	106	\pm 13
6	96	\pm 24	106	\pm 19
8	102	\pm 16	99	\pm 7
9	105	\pm 14	104	\pm 17

OPERATIVE ANIMALS								
ANIMAL NO.	PREOPERATIVE PERIOD		POSTOPERATIVE PERIOD					
	MEAN	STAND. DEV.	1†	2	3	4	5	6
3	110	\pm 13	22	39	55	80	104	127
5	103	\pm 17	36	35	46	42	66	83
1	109	\pm 15	364	148	82	77	67	63
7	97	\pm 15	132	105	111	89	133	127
								131

*Sugar is expressed in milligrams per 100 c.c.

†Number of days after operation.

Rat No. 5 also showed a marked and significant decrease in the blood sugar, from a preoperative mean of 103 mg. \pm 17 mg. per 100 c.c. of blood to 36 mg. per 100 c.c. of blood. On the seventh postoperative day the blood sugar was definitely in the preoperative level.

Rat No. 1 demonstrated a marked hyperglycemia. The blood sugar rose to 364 mg. per 100 c.c. of blood the first day postoperatively. Subsequent readings declined rapidly. By the seventh postoperative day the readings had returned to the preoperative level.

Rat No. 7 demonstrated no significant blood sugar changes throughout the course of the experiment.

Histologic Studies.—Sections were made of the liver, pancreas, adrenals, spleen, and testicles of all eight rats. No abnormal changes were found. The size, shape, and number of the islands of Langerhans of the operative animals were similar to those of the controls.

COMMENTS AND CONCLUSIONS

The postoperative blood sugars of rats Nos. 1 and 7 rule out the possibility that surgical trauma per se was responsible for the changes in the blood sugar levels. Decreased daily feedings or loss of weight alone could not have been the cause for these changes, since all four operative animals lost weight. Only the rats totally parotidectomized manifested significant blood sugar drops. We may conclude that these drops were directly due to the removal of a substance in the parotid gland which acts antagonistically to the action of insulin. Furthermore, the presence of even a minute fragment of parotid gland will prevent this fall in blood sugar, as sufficient antagonist can be elaborated by

such small fragments as were left in rat No. 7. Rat No. 1, on the other hand, demonstrated what may be considered an oversecretion of the parotid gland, due to irritations caused by ligatures passed through the gland. Subsequent tissue studies of the remnant of parotid gland in rat No. 1 revealed no structural changes.

We may conclude that the absence of parotid gland definitely lowers the blood sugar levels and, conversely, that the parotid secretes a substance that elevates the blood sugar.

Cahane and Cahane's⁴ findings of hyperplasia and hypertrophy of the islands of Langerhans were not confirmed.

The significance of the reports of Aunaps⁵ concerning the cure of hyperglycemia following parotidectomy of depancreatized dogs must remain in doubt unless a compensatory hypertrophy of unremoved pancreatic tissue in his animals can be ruled out.

The nature of the compensatory mechanism effecting the return of blood sugars to the preoperative levels is not yet understood. Further work along these lines is indicated.

SUMMARY

1. The blood sugars decreased markedly, though temporarily, in totally parotidectomized rats.
2. The blood sugar was not affected when approximately one-twelfth of the total weight of both glands was left in situ.
3. The blood sugar increased markedly, though temporarily, following ligation of part of the parotid gland.
4. All blood sugars returned to preoperative levels eight days following the operative procedure.
5. Histologic studies of the liver, pancreas, adrenals, spleen, and testicles of the deparotidectomized rats revealed no changes.

REFERENCES

1. Hiki, Y., Akazaki, T., Miyazaki, Y., and Takazawa, N.: Experimentelle Untersuchungen der Mundspeicheldrüsen, *Tr. Jap. Path. Soc.* 22: 273, 1932.
2. Takacs, L.: Internal Secretion of the Parotid Gland, *Orvosi hetil.* 77: 659, 1933.
3. Rosenfeld, G.: Speicheldrüsen Kohlehydratstoffwechsel, *Klin. Wchnschr.* 12: 711, 1933.
4. Cahane, M., and Cahane, T.: Sur un cas de diabète sucré avec hypertrophie des Glandes Parotides, *Rev. franç. d'endocrinol.* 11: 336, 1933.
5. Aunap, E.: Ueber die Beziehungen zwischen Kern, Ergastoplasma und Mitochondrien der Parotiszellen der Ratte, *Ztschr. f. mikr.-anat. Forsch.* 24: 419, 1931.
6. Ferretti, G.: Su due casi di ipertrofia delle parotidi in diabetici, *Gior. di clin. med.* 17: 1305, 1936.
7. Scott, E. L.: Reducing Power (Blood Sugar) of Filtrates From Blood of Rabbits, *Arch. Int. Med.* 43: 393, 1929.
8. Benedict, S. R.: The Analysis of Whole Blood. 2. The Determination of Sugar and of Saccharoids (Nonfermentable Copper-Reducing Substances), *J. Biol. Chem.* 92: 141, 1931.

SERUM CHOLESTEROL STUDIES FOLLOWING THE ADMINISTRATION OF ACETYL- β -METHYLCHOLINE CHLORIDE BY IONTOPHORESIS*

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THAT choline is important in cholesterol metabolism has been pointed out by Best and his coworkers¹⁻³ and by other workers. Best has shown that the administration of choline prevents the fatty infiltration of the liver in rats fed fat or cholesterol. Steiner,⁴ working with 38 rabbits, some of which received cholesterol and others cholesterol and choline, found that the oral administration of 0.5 Gm. of choline daily to cholesterol-fed rabbits did not prevent the development of hypercholesterolemia when compared to the control group receiving cholesterol alone. He also concluded from this study that choline delayed, but did not prevent, experimental atherosclerosis. Baumann and Rusch⁵ also observed that choline did not influence the cholesterol content of the blood in cholesterol-fed rabbits. Zinnitz⁶ found that small amounts of cholesterol acetate inhibited the action of acetylcholine.

During the routine treatment of patients with acetyl- β -methylcholine chloride by iontophoresis, it appeared that a study of the serum cholesterol levels before and after treatment might be of interest. Accordingly, 12 persons were selected; 2 received 0.125 per cent acetyl- β -methylcholine chloride by iontophoresis and the remaining 10, 1 per cent of the drug by the same route. Serum cholesterol determinations were done before and at intervals of one-half, one, and two hours after the administration of the drug. Since the effect of the acetyl- β -methylcholine chloride occurs within a few minutes and persists for several hours, it was felt that the time intervals selected were of sufficient duration to demonstrate an effect, if any. Cholesterol determinations were done in duplicate by a modified Bloor's method.^{7, 8}

RESULTS

Of the 2 persons receiving 0.125 per cent acetyl- β -methylcholine chloride, one showed an appreciable drop in serum cholesterol in one-half hour, with a marked rise in the one- and two-hour specimens, while the other showed a slight rise from the control value. Five of the ten persons receiving the 1 per cent solution showed an appreciable rise in serum cholesterol, varying from 11 to 27 mg. above the control levels at the end of the two-hour period. Of the remaining 5, 2 showed no change, while 3 showed a slight fall in blood cholesterol. The serum cholesterol in the 12 persons studied varied by an average standard deviation of ± 5.1 per cent, with a range of ± 1.9 to ± 9.7 (Table I).

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TABLE I

SUBJECT	SERUM CHOLESTEROL (MG. PER 100 C.C.)				MEAN	STANDARD DEVIATION (\pm %)	ACETYL- β - METHYL- CHOLINE (%)
	CONTROL	$\frac{1}{2}$ HR.	1 HR.	2 HR.			
D. B.	222	189	218	242	218	9.5	0.125
A. S.	200	214	211	208	208	2.6	0.125
A. C.	240	257	295	268	265	9.7	1
I. K.	218	195	216	236	216	7.2	1
R. McC.	170	169	175	190	176	4.2	1
L. C.	242	239	230	244	239	2.7	1
L. C.	229	259	248	240	244	5.5	1
A. H.	164	168	160	170	166	1.9	1
A. J.	160	140	150	152	151	3.5	1
M. B.	293	259	286	280	280	6.2	1
J. G.	272	268	286	291	279	4.5	1
C. G.	174	157	171	174	169	3.2	1
Average						± 5.1	

DISCUSSION

That acetyl- β -methylcholine chloride exerts a physiologic action when given by iontophoresis has been amply demonstrated by Kovacs, Saylor, and Wright,⁹ and by Kotkis and others.¹⁰ These workers have demonstrated that the cations of acetyl- β -methylcholine chloride were responsible for the physiologic reaction due to this compound and that they could be driven into the body by iontophoresis only from the positive electrode of a galvanic source.

Although rises and falls occurred in serum cholesterol following acetyl- β -methylcholine chloride iontophoresis, on analysis these variations were found to lack statistical significance. Bruger and Somaeh,¹¹ reporting on the diurnal variations in blood cholesterol in a group of 18 normal persons, found the average standard deviation to be ± 5.0 per cent for a twenty-four-hour period, with an average ± 3.5 per cent standard deviation in the morning hours. Since the present experiments were carried out during the morning hours, the average standard deviation of ± 5.1 per cent found in this series was only slightly higher than the ± 3.5 per cent reported by Bruger and Somaeh.¹¹

CONCLUSIONS

The administration of acetyl- β -methylcholine chloride by iontophoresis is accompanied by variations of the blood cholesterol, which, however, are not significant from a statistical standpoint.

REFERENCES

1. Best, C. H., and Huntsman, M. E.: Effects of Components of Lecithine Upon Deposition of Fat in Liver, *J. Physiol.* 75: 405, 1932.
2. Best, C. H., Ferguson, G. C., and Hershey, J. M.: Choline and Liver Fat in Diabetic Dogs, *J. Physiol.* 79: 94, 1933.
3. Best, C. H., and Ridout, J. H.: Effect of Cholesterol and Choline on Liver Fat, *J. Physiol.* 86: 343, 1936.
4. Steiner, A.: Effect of Choline on Production of Experimental Atherosclerosis in Rabbits, *Proc. Soc. Exper. Biol. & Med.* 38: 231, 1933.
5. Baumann, C. A., and Rusch, H. P.: Effect of Choline on Atherosclerosis in Rabbit Aorta, *Proc. Soc. Exper. Biol. & Med.* 38: 647, 1938.
6. Zinnitz, F.: Acetylcholin und Cholestein, *Arch. f. exper. Path. u. Pharmacol.* 190: 594, 1938.
7. Sackett, G. E.: Modification of Bloor's Method for Determination of Cholesterol in Whole Blood or Blood Serum, *J. Biol. Chem.* 64: 203, 1925.

8. Mirsky, I. A., and Bruger, M.: Note on Liebermann-Burchard Color Reaction for Cholesterol, *J. LAB. & CLIN. MED.* 18: 304, 1932.
9. Kovacs, J., Saylor, L. L., and Wright, I. S.: Pharmacological and Therapeutic Effects of Certain Choline Compounds; Results in Treatment of Hypertension, Arthritis, Organic Occlusive Vascular Disease, Raynaud's Disease, Scleroderma and Varicose Ulcers, *Am. Heart J.* 11: 53, 1936.
10. Kotkis, A. J., and Melchionna, R. H.: Physiologic Effects of Acetyl-beta-methylcholine Chloride by Iontophoresis, Preliminary Report, *Arch. Phys. Therapy* 16: 528, 1935.
11. Bruger, M., and Somach, I.: Diurnal Variations of Cholesterol Content of Blood, *J. Biol. Chem.* 97: 23, 1932.

THE DISTRIBUTION OF DOSES OF RADIOACTIVE PHOSPHORUS IN RODENTS*

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WITH growing interest in the therapeutic uses of temporarily radioactive substances, it is important to learn the distribution of these in tissue and their rate of excretion. Radioactive phosphorus prepared† at the Harvard cyclotron by bombardment of red phosphorus was injected intravenously or intraperitoneally in mice, rats, and rabbits as aqueous solution of disodium acid phosphate. The dosages of radioactive material injected ranged from 5 microcuries to 17.5 microcuries in the mouse, from 30 microcuries to 239 microcuries in the rat, and from 50 microcuries to 84 microcuries in the rabbit.

Measurements were made by a Lauritsen type electroscop. The accuracy of measurements hinges on (a) the rate of deflection of the fiber on any given portion of the scale, and (b) the human error in determining the exact positions of the fiber in relation to the scale markings. This last error increases as the rate of deflection decreases. Hence values obtained for relatively active samples are more accurate than are those for samples of very low activity. The thickness of the aluminum foil window is of importance in the determinations. Ours is 0.0055 mm. thick, permitting passage of about 75 per cent of the soft β radiation of uranium oxide. The sensitivity of this electroscop is two divisions per minute per millicurie of radiation at one meter distance. We do not regard as accurate our measurements below 0.01 microcurie, although phosphorus of this degree of radioactivity may be detected in amounts of 3.5×10^{-14} Gm. About 10 per cent error must be allowed in all results by this method of measurement.

Although expressed in microcurie equivalents, the radiation is not actually comparable in biologic effect to the same amount of gamma radiation of radon. The tissue changes are now being studied.

*From the Laboratories of Pathology of the New England Deaconess and Collis P. Huntington Hospitals, and the Department of Pathology of Harvard Medical School, Boston.
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Radioactive phosphorus decays with a half life of 14.5 days. The radiation is made up of β particles,¹ having a half layer value of 0.5 mm. of aluminum.²

TABLE I
PER CENT PARTITION OF RADIOACTIVE PHOSPHORUS IN RATS

HOURS	2	48	120	168
Heart	0.3	0.4	0.3	0.3
Lung	0.1	1.2	0.3	0.6
Spleen	0.1	0.6	0.7	0.4
Liver	0.2	8.7	5.0	2.8
Kidneys	0.3	0.5	0.4	3.1
Bone	0	11.1	11.3	4.0

TABLE II
PER CENT PARTITION OF RADIOACTIVE PHOSPHORUS IN RABBITS

HOURS	24	48	120	168	240	2,592
Heart	0.6	0.6	0.7	0.4	0	0
Lungs	1.0	1.0	1.3	0.5	0	0
Spleen	1.4	0.4	0.2	0.2	0	0
Liver	11.1	11.4	9.1	5.3	0	0
Kidneys	0.7	0.6	0.7	0.6	0	Trace
Bone	1.4	0	0	0	0	Trace
(2 femurs)						
Blood	2.8	2.6	0.14	0.13	Trace	0
(total)						

TABLE III
PER CENT PARTITION OF RADIOACTIVE PHOSPHORUS IN MICE

HOURS	24	48	120	240	264	312
Heart	0.35	0.35	0.3	0.24	0.12	0.15
Lungs	0.7	0.7	0.4	0.2	0.24	0.2
Spleen	1.0	1.5	0.6	0.3	0.2	0.1
Liver	4.9	4.1	2.1	1.0	1.1	0.6
Kidneys	1.0	0.0	0.5	0.4	0.3	0.3

TABLE IV
URINARY EXCRETION OF RADIOACTIVE PHOSPHORUS IN THE RAT

HOURS	PER CENT
3	6.1
6	6.3
21	5.6
30	3.5
45.5	2.9

In Tables I, II, III, and IV the amounts of radioactive phosphorus present in the tissues are calculated in per cent of the dose originally injected, corrected for rate of decay. In the case of larger tissues measurements were made of weighed aliquot portions; in the case of smaller organs the entire organ was weighed and used. Blood and urine were dried before measurement,* other tissues were compressed to a thin film and dried, or ground and then dried, or ashed.

As may be seen from Tables I to III, the per cent partition varies in the different animals. These values are only indicative of general trends, and vary

*The addition of varying amounts of urine up to fivefold, causing variation in thickness of the dry films, did not affect the accuracy of measurement.

somewhat with dosages given as well as other factors. Some absorption occurs within two hours after injection (Table I). The bone naturally picks up a large amount and retains it long, traces being present in the rabbit after 108 days. Owing probably to their excretory activity, the kidneys retained a trace at the same time, although none could be found in the liver (Table II).

The partition varies in the different species, but in general the spleen, liver, kidneys, and bone show a material degree of absorption and retention. In view of the distribution of leucemic lesions, this partition is serviceable in concentrating radioactivity to some degree in the tissues often affected by leucemic infiltration.

Urinary excretion is rapid. Hevesy³ found when tracer quantities of radioactive sodium phosphate are given by mouth, traces appear in the urine in twenty minutes. This urinary excretion is more marked early and falls off rapidly. About one-quarter the dose is excreted in the first forty-eight hours. Minute amounts were found in the urine of a rat ninety-eight days after subcutaneous injection.³

Table IV gives the rate of urinary excretion of radioactive phosphorus administered intraperitoneally or intravenously. In the first three hours, 6 per cent is excreted, and this rate continues with only slight diminution for twenty-one hours, falling to about one-half in the next day.

The fecal excretion could not be accurately measured in our animals, as slight contamination with urine could not be prevented. Tuttle, Scott, and Lawrence⁴ found from 13 per cent to 26 per cent of orally administered radioactive phosphorus in the feces of leucemic patients. An unknown amount of this represents failure of absorption from the gastrointestinal tract.

There is variation in the per cent partition in various tissues, to a minor degree among individual animals of the same species and to a greater degree between different species.

REFERENCES

1. Rasetti, F.: *Elements of Nuclear Physics*, New York, 1936, Prentice-Hall.
2. Hevesy, G.: *J. Chem. Soc.* p. 1213, 1939.
3. Hevesy, G.: *Det. Kgl. Danske Videnskab. Selskab. Biol. Medd.* 14: 3, 1939.
4. Tuttle, L. W., Scott, K. G., and Lawrence, J. H.: *Proc. Soc. Exper. Biol. & Med.* 41: 29, 1939.

THE ESTER-HYDROLYZING ACTIVITY OF THE CENTRAL NERVOUS SYSTEM*

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INTRODUCTION

LIPIDS such as cephalin, sphingomyelin, lecithin, cholesterol, and related compounds form a major portion of nervous tissue. In the human brain and spinal cord, for example, fatty materials constitute about 50 per cent of the dry weight of the tissue,^{1, 2} as compared with 15 per cent in muscle tissue.³ It follows that lipid metabolism is of profound interest in the study of the biochemical processes that take place in the brain and nerves. Marked changes are known to occur in the compounds composing the myelin sheaths, especially during nerve formation and degeneration.^{1, 4}

Naturally, lipolytic and ester-splitting enzymes may be expected to play an important part in the formation and transformation of nerve tissue lipids. Bearing out this deduction is the increase in lipolytic enzyme activity of the cerebrospinal fluid found in conditions in which severe brain tissue destruction is known to occur.⁵

Despite the fact that lipolytic enzymes may thus be expected to be present in nervous tissue in considerable amounts, earlier workers did not find appreciable lipolytic activity.⁶⁻¹⁰ We surmised that this was due to the use of tissue extracts or autolysates. In the first instance they missed the desmoenzymes firmly attached to cellular structures; in the second, part of the enzymes were inactivated during autolysis.¹¹ We, therefore, used minced brain tissue carefully guarded against autolysis. By this technique we have been able to demonstrate marked lipolytic activity.

In this report we are presenting the results of quantitative estimations of the ester-splitting activity of various portions of the brain and spinal cord. This "enzyme map" of nervous tissue is of itself interesting in that the lipolytic activity has been found to vary with the anatomic structures and possibly, in turn, with the functions of the tissue. However, the main function of these normal distribution studies is to provide a standard of comparison for studies of diseased nervous tissue which are in progress.

METHODS

Preparation of the Tissue.—Throughout the experiments the brain and spinal cord of the monkey, *Macaca mulatta*, were used.† The monkeys had died of either

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pneumonia or tuberculosis so that they were not, strictly speaking, normal animals. However, the brains and cords appeared normal on gross dissection. In doubtful cases microscopic sections were made, but in no cases were abnormalities found. As soon as possible after death the brain and cord were removed and washed in a stream of distilled water to remove blood adhering to the surface. After stripping the leptomeninges, the cerebrum and cerebellum were separated from the pons and medulla oblongata. The following tissues were removed as carefully and completely as possible: cerebral cortex, centrum semiovale, corpus callosum, thalamus, and gray tissue of the caudate and lenticular nuclei. A slice of cerebral tissue (mixed cerebrum) containing cortex and subcortical white matter was also removed. No attempt was made to separate gray and white matter of the cerebellum, pons, or medulla, since the small size of the monkey brain made this impractical. In this study the pons and medulla, which were always combined, are referred to as the brain stem.

The tissues were minced separately in Petri dishes with a scalpel and spatula, formed into oblong cakes, and kept in the refrigerator at 0° to 5° C. Precautions were taken to minimize the length of time during which any tissue was exposed to room temperature. Tissue prepared in this way could be kept for a week or more with no significant loss of lipolytic activity.

Determination of the Lipolytic Activity.—The lipolytic activity was determined as follows: Substrate emulsions of tributyrin, ethyl butyrate, or olive oil, were prepared by shaking one part of substrate with 4 parts of 5 per cent gum acacia solution. The hydrolysis mixtures, made up in 50 c.c. Erlenmeyer flasks, preferably glass stoppered, contained 2 c.c. of substrate emulsion, 1 c.c. or more of buffer solution, and enough distilled water to bring the volume to 10 c.c. The amount and concentration of the buffer solution depend on the purpose of the experiment. A piece of tissue cut from the previously described cake and estimated to weigh between 250 and 300 mg. was used as the enzymic material. The exact moist weight of tissue was formed by weighing the hydrolysis flask before and after addition of the tissue. The flask was shaken vigorously by hand until the tissue was distributed in small pieces through the emulsion, and then incubated for twenty-four hours at 37° C. At the end of this period the reaction was stopped by the addition of 10 c.c. of 95 per cent alcohol, and the amount of acid formed was determined by titration with 0.05 normal sodium hydroxide, using 10 drops of 1 per cent phenolphthalein as indicator. Two kinds of control determinations were used. A substrate blank was determined by titrating a hydrolysis mixture after incubation without brain tissue. A tissue blank was determined by incubating tissue with buffer and water without substrating and titrating as previously the lipolytic activity was calculated as follows:

(1) Lipolytic activity per gram of tissue

$$= \left[\frac{\begin{array}{cc} \text{(c.c. 0.05 N NaOH)} & \text{—(c.c. 0.05 N NaOH)} \\ \text{Tissue and buffered} & \text{Buffered} \\ \text{substrate} & \text{substrate} \end{array}}{\text{(weight of tissue used, in milligrams)}} \right] \times 1000$$

—Autolysis value per gram of tissue.

The "autolysis value" in the foregoing formula was obtained as follows:

(2) Autolysis value per gram of tissue

$$= \left[\frac{(\text{c.c. of 0.5 N NaOH}) - (\text{c.c. 0.05 N NaOH})}{\frac{\text{Tissue and buffer}}{(\text{weight of tissue used, in milligrams})} \times \frac{\text{Buffer}}{1}} \right] \times 1000$$

Throughout the course of the experiments the phosphate buffer of Sørensen, as modified by Hastings and Sendroy,¹² was used. In order to avoid the arbitrariness involved in the use of a single buffer concentration, we studied the lipolytic activity at various concentrations of phosphate buffer. A stock solution of buffer of pH 7.9 was prepared at a salt concentration of 0.2 molar. By using 1, 2.5, 4, 5, or 8 c.c. of this stock solution in hydrolysis mixtures whose total volume was 10 c.c., it was possible to obtain phosphate buffer concentrations of 0.02, 0.05, 0.08, 0.10, and 0.16 molar, respectively. To obtain a buffer concentration of 0.0067 molar, 1 c.c. of fifteenth-molar stock solution was used. The activity was determined at pH 7.9 because this pH value is in the neighborhood of maximum hydrolysis of tributyrin and ethyl butyrate. Experiments were also made at the more physiologic pH value of 7.4. The difference in pH exerted no significant influence on the results obtained.

In all, 13 monkeys were used in the experiments reported. In view of the fact that the results obtained with individual animals showed relatively small variations, this number was considered sufficient.

RESULTS

All portions of nervous tissue tested showed considerable tributyrin and ethyl butyrate-splitting activity. This activity per gram of material was of the order of ten times that of blood serum. Neutral fat (olive oil) was not appreciably hydrolyzed.

The concentration of phosphate buffer in the hydrolysis mixture affected the rate and extent of hydrolysis. Within certain limits increased phosphate buffer concentration was accompanied by increased hydrolysis. In the case of ethyl butyrate this increase reached a maximum at 0.02 to 0.05 molar phosphate buffer concentration. Tributyrin-splitting activity reached a maximum at 0.16 molar.

Although the absolute ester-splitting power, as represented by the titration values in Table I and Figs. 1 and 2, varied from animal to animal, the relative order in which tissues from each individual animal split each ester was always the same. In other words, the ester-splitting power of the different tissues was characteristic of the tissues; the relative hydrolyzing power of various tissues under similar conditions of hydrogen-ion concentration, temperature, and phosphate buffer concentration was always the same. Thus at 0.16 molar phosphate buffer concentration the various tissues studied hydrolyzed tributyrin in the following order: cerebral cortex, mixed cerebrum, striatal gray matter, thalamus, cerebellum, stem, spinal cord, and white matter (Fig. 1). At lower phosphate buffer concentrations the difference in the tributyrin-splitting activity of the several tissues was not quite so distinct, yet gray and mixed tissues always showed more tributyrin-splitting activity than spinal cord and white matter,

TABLE I

HYDROLYSIS OF TRIBUTYRIN AND ETHYL BUTYRATE BY VARIOUS TISSUES OF THE CENTRAL NERVOUS SYSTEM

(Results are expressed as cubic centimeters of 0.05 normal sodium hydroxide used to neutralize the fatty acids formed.)

		CERE- BRAL CORTEX	STRIATAL GRAY TISSUE	MIXED CERE- BRUM	THALA- MUS	CERE- BELLUM	STEM	SPINAL CORD	WHITE MATTER
0.16 Molar Phosphate Buffer									
Tributyrin	Max.	100.0	101.0	94.1		79.4	68.6	61.2	54.9
	Min.	82.7	72.9	82.6		76.8	51.5	48.5	46.8
	Avg.	92.3	85.3	89.4	80.0	73.7	60.8	53.8	50.7
Ethyl butyrate	Max.	25.7	26.0	24.9	23.8	19.0	18.7	11.2	13.0
	Min.	20.9	14.0	14.4	13.6	8.5	12.3	6.1	6.8
	Avg.	23.1	19.0	20.2	18.7	13.5	15.1	7.8	10.8
0.10 Molar Phosphate Buffer									
Tributyrin	Max.	80.2	70.0	73.4	64.1	71.3	58.1	54.0	51.8
	Min.	68.7	52.5	63.1	57.0	58.7	43.1	45.0	33.5
	Avg.	75.4	63.6	68.4	60.5	64.9	51.5	48.8	44.3
Ethyl butyrate	Max.	28.4	25.2	23.4	20.7	19.2	20.4	11.7	13.9
	Min.	21.0	15.7	17.0	16.7	10.2	9.6	6.6	7.4
	Avg.	23.6	20.6	20.0	18.7	14.4	15.0	8.5	11.0
0.0067 Molar Phosphate Buffer									
Tributyrin	Max.	14.9	15.7	17.1	17.3	16.0	16.3	13.2	14.6
	Min.	13.2	11.6	13.2	12.6	12.2	13.9	10.8	10.9
	Avg.	14.4	14.0	14.7	14.1	14.4	15.2	12.3	11.9
Ethyl butyrate	Max.	9.1	11.3	9.3	10.2	9.8	7.1	6.0	7.6
	Min.	6.6	6.9	5.6	7.0	4.5	5.7	3.0	3.4
	Avg.	8.2	8.6	7.3	7.9	6.5	6.5	4.4	5.3

Ester-splitting power of nervous tissue is strongest in gray matter. Phosphate buffer concentration has a notable effect.

On the other hand, ethyl butyrate-splitting activity at all phosphate concentrations remained in the order: cerebral cortex, mixed cerebrum, striatal gray matter, thalamus, stem, cerebellum, white matter, and spinal cord (Fig. 2).

It will be noted that the order of ethyl butyrate-splitting activity is somewhat different from that for tributyrin-splitting activity. The stem is more active than the cerebellum with respect to ethyl butyrate, and white matter is more active than spinal cord tissue. These differences were found consistently in all the animals studied.

DISCUSSION

It has been found in the present work that all the various tissues of the brain and spinal cord which we have studied have marked ester-splitting ability. The quantitative measure of this ester-splitting activity varies throughout the central nervous system and is a chemical characteristic of each tissue portion. In general, the ester-splitting activity is greatest in gray matter, least in white matter, and intermediate in mixed tissues.

The presence of lipolytic enzymes in the brain and spinal cord raises the question of the source and functions of these enzymes. Since white matter consists of nerve fibers and neuroglial cells, the enzymes of white matter are probably derived from the latter. Whether or not astrocytes, oligodendroglia, and microglia, contribute equally to the enzymatic activity of the white matter, is a question which cannot at present be settled. It is thought, on anatomic grounds,¹³ that the oligodendroglia are related to the formation and maintenance

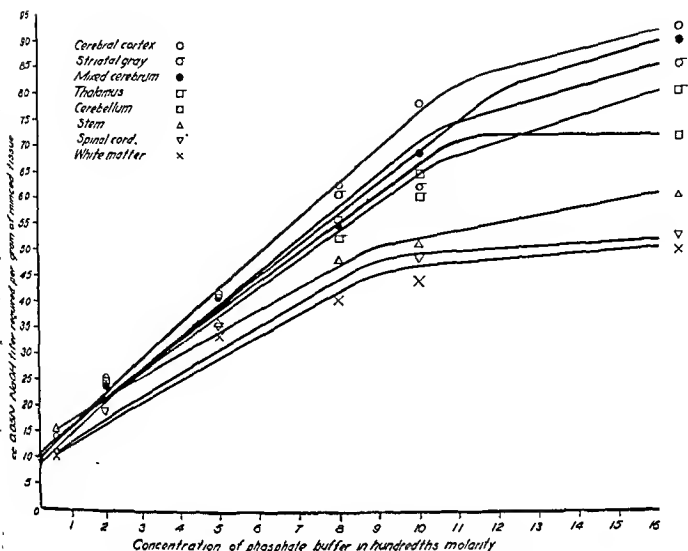


Fig. 1.—Curves showing the hydrolysis of tributyrin by various central nervous system tissues at different concentrations of phosphate buffer.

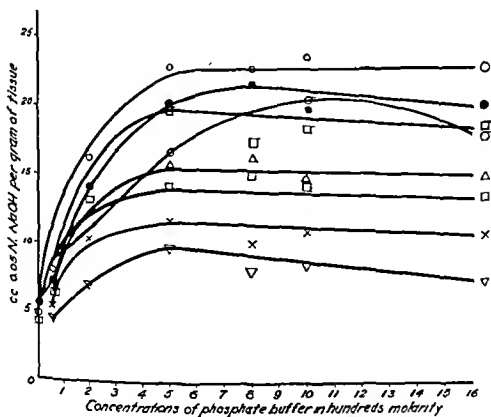


Fig. 2.—Curves showing the hydrolysis of ethyl butyrate by various central nervous system tissues at different concentrations of phosphate buffer. See Fig. 1 for key.

TABLE I

HYDROLYSIS OF TRIBUTYRIN AND ETHYL BUTYRATE BY VARIOUS TISSUES OF THE CENTRAL NERVOUS SYSTEM

(Results are expressed as cubic centimeters of 0.05 normal sodium hydroxide used to neutralize the fatty acids formed.)

		CERE- BRAL CORTEX	STRIATAL GRAY TISSUE	MIXED CERE- BRUM	THALA- MUS	CERE- BELLUM	STEM	SPINAL CORD	WHITE MATTER
Tributyrin	Max.	100.0	101.0	0.16	Molar Phosphate			Buffer	
	Min.	82.7	72.9	94.1		79.4	68.6	61.2	54.9
	Avg.	92.3	85.3	82.6		76.8	51.5	48.5	46.8
Ethyl butyrate	Max.	25.7	26.0	89.4	80.0	73.7	60.8	53.8	50.7
	Min.	20.9	14.0	24.9	23.8	19.0	18.7	11.2	13.0
	Avg.	23.1	19.0	14.4	13.6	8.5	12.3	6.1	6.8
				20.2	18.7	13.5	15.1	7.8	10.5
Tributyrin	Max.	80.2	70.0	0.10	Molar Phosphate			Buffer	
	Min.	68.7	52.5	73.4	64.1	71.3	58.1	54.0	51.8
	Avg.	75.4	63.6	63.1	57.0	58.7	43.1	45.0	33.5
Ethyl butyrate	Max.	28.4	25.2	68.4	60.5	64.9	51.5	38.8	44.3
	Min.	21.0	15.7	23.4	20.7	19.2	20.4	11.7	13.9
	Avg.	23.6	20.6	17.0	16.7	10.2	9.6	6.6	7.4
				20.0	18.7	14.4	15.0	8.5	11.0
Tributyrin	Max.	14.9	15.7	0.0067	Molar Phosphate			Buffer	
	Min.	13.2	11.6	17.1	17.3	16.0	16.3	13.2	14.6
	Avg.	14.4	14.0	13.2	12.6	12.2	13.9	10.8	18.9
Ethyl butyrate	Max.	9.1	11.3	14.7	14.1	14.4	15.2	12.3	11.9
	Min.	6.6	6.9	9.3	10.2	9.8	7.1	6.0	7.6
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matter, such as thalamus and cerebellum, were more active than mixed tissues rich in white matter, such as stem and spinal cord.

4. The ethyl butyrate-splitting activity was greatest in the cerebral cortex and least in the spinal cord. The cerebellum has less activity than other mixed tissues including the stem. White matter was less active than gray or mixed tissues with the exception of the spinal cord.

REFERENCES

1. Page, I. H.: *Chemistry of the Brain*, Springfield, Ill., 1937, C. C. Thomas.
2. Randall, L. O.: *Chemical Topography of the Brain*, J. Biol. Chem. 124: 481, 1938.
3. Bodansky, M.: *Introduction to Physiological Chemistry*, ed. 3, New York, 1934, John Wiley & Sons, p. 609.
4. Weil, A.: *A Textbook of Neuropathology*, Philadelphia, 1933, Lea & Febiger.
5. Kaplan, I., Colm, D. J., Levinson, A., and Stern, B.: Sources of the Enzymes of Normal and Pathologic Cerebrospinal Fluid, J. LAB. & CLIN. MED. 25: 495, 1940.
6. English, H. M., and MacArthur, C. G.: Enzymes of the Central Nervous System, J. Am. Chem. Soc. 37: 653, 1915.
7. Quinan, C.: Lipase Studies. IV. On Regional Lipolytic Activity in the Normal Human Brain, J. M. Research 35: 79, 1916.
8. Sugiyama, K., Noyes, H. M., and Falk, K. G.: Comparative Lipase and Protease Actions of Different Beef Tissues, J. Biol. Chem. 59: 213, 1924.
9. Hiller, F.: Die Beziehungen der degenerativen Veränderungen des Zentralnervensystems zu seinem Gehalt an Fett und Ester spaltenden Enzymen; sowie der Nachweis sowohl dieser Enzyme als auch von Lipoidstoffwechselschlacken des Nervengewebes im Liquor cerebrospinalis, Ztschr. f. d. ges. Neurol. u. Psychiat. 109: 263, 1927.
10. Takasaka, T.: Ueber den Fermentgehalt des menschlichen Gehirns. Biochem. Ztschr. 184: 390, 1927.
11. Unpublished observations.
12. Clark, W. M.: *The Determination of Hydrogen Ions*, Baltimore, 1928, Williams & Wilkins Co., p. 212.
13. Penfield, W.: *Neuroglia: Normal and Pathological, Cytology and Cellular Pathology of the Nervous System*, New York, 1932, Paul B. Hoeber, Vol. 11, p. 423.

LABORATORY METHODS

GENERAL

A NOTE ON THE BACTERIOLOGIC CULTURE OF BONE MARROW IN TYPHOID FEVER*

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THE localization of bacilli in the bone marrow during an attack of typhoid fever has been known for many years. Lougcope¹ in 1905 noted the presence of large numbers of characteristic macrophages here, as well as in the Peyer's patches of the intestines, and also observed areas of necrosis in the marrow similar to those of the spleen and lymph nodes. Ludke² reported the persistence of typhoid bacilli in the lymph nodes and bone marrow of guinea pigs following intravenous inoculation and their proliferation in these situations after disappearance from the blood. Gay³ further noted that the organisms were present in the marrow of infected rabbits not only after disappearance from the blood, but also from the lymph nodes and spleen. The clinical significance of this localization has been stressed in regard to the resultant leucopenia and neutropenia occurring during the acute phase of the disease and the osseous lesions which occasionally occur as sequelae of the infection. However, the value of marrow culture during the disease has been pointed out only within the past several years. In this communication we wish to record our experience with the bacteriologic examination of the marrow in three cases of typhoid and to comment briefly on the usefulness of this procedure.

Culture of the bone marrow *in vivo* has been closely associated with the increasing interest and exploitation of bone marrow biopsy in the blood dyscrasias. Cytologic examinations of the marrow have increased in number as a result of the introduction of simpler and more practical methods of securing samples of this tissue. Among the earliest attempts at marrow biopsy were those of Ghedini,⁴ who first advocated tibial trephination for the diagnosis of leishmaniasis. This technique never became widely popular, although it was used by Zadek⁵ and Peabody⁶ in 1922 and 1927, respectively, in their classical studies on the marrow changes in pernicious anemia. Sternal trephination, introduced by Seyfarth⁷ in 1923, reawakened interest in the diagnostic value of marrow biopsy. But it remained for the introduction of needle puncture of the sternum by Arinkin⁸ in 1929 to popularize marrow study.

Within the past several years reports of attempts at bacteriologic and parasitologic examination of marrow in a variety of infections have appeared.

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largely in European literature. Among the diseases so studied have been typhoid fever, brucellosis, subacute bacterial endocarditis, miliary tuberculosis, malaria, leishmaniasis, trypanosomiasis, and various septic states due to members of the pyrogenic group of bacteria. Marrow examination in leishmaniasis seems to be accepted as a diagnostic procedure of recognized value.⁹ In malaria Osgood¹⁰ has reported the occurrence of larger numbers of parasites in the marrow than in the peripheral blood. Bock¹¹ concluded from a comparative study of arterial and venous blood cultures with marrow culture in a variety of septic states that the latter, while inferior to arterial culture, was considerably easier to obtain and was apparently superior to the more commonly used venous blood culture. Favorable experiences with this method in the diagnosis of brucellosis have been reported by Signorelli¹² and Baserga and Barbagallo.¹³

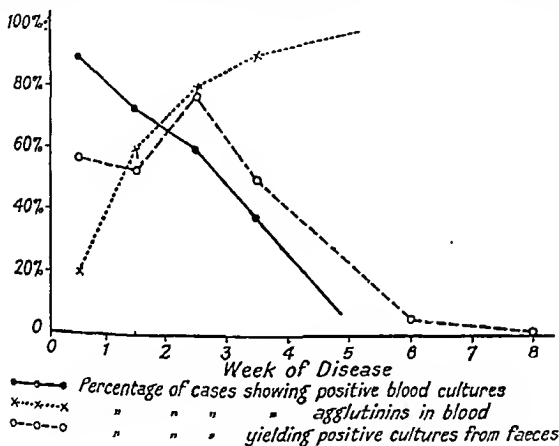


Fig. 1.
(Courtesy of Topley and Wilson)

The earliest report of bacteriologic culture of bone marrow biopsy material in typhoid fever is contained in the little-known paper of Gerbasi,¹⁴ who in 1925 obtained positive results from tibial bone marrow in thirteen cases. No further reports occurred until 1935, when Debré and co-workers¹⁵ cultured the marrow obtained by sternal puncture in ten cases of typhoid during the first and second weeks of infection and obtained positive results in all instances. Further studies are recorded by Storti and Filippi,¹⁶ Baserga and Barbagallo, and others. In all, approximately two hundred cases are included in these reports. Positive cultures were apparently obtained from patients not only in the first few days of the disease, but also as late as sixty to sixty-five days following the onset. It would appear from a scrutiny of this material that typhoid bacilli are present over a longer period of time in the marrow than in any other situation and that this tissue offers a constant reservoir from which positive cultures may be obtained.

The aim of diagnosis in typhoid fever has always been the actual isolation and identification of the organism from any possible source. The most fruitful sources for culture in active cases have been the blood and feces. Determination of the agglutinin content of the serum is an indirect method of diagnosis, the reliability of which depends on the demonstration of progressive increase in titer. Frequently titers of sufficient strength to be diagnostic do not develop before the end of the second week of the illness. Moreover, in this day of increasingly widespread use of prophylactic vaccination, the possibility of an "anamnesic reaction" of typhoid agglutinins to nonspecific hyperpyrexia must always be considered. Reference to Fig. 1 demonstrates that during the latter part of the second week of illness the diagnostic efficiency of current laboratory measures averages about 70 per cent. During this time, which has been called the "mute period" by Baserga and Barbagallo, marrow culture appears to have its greatest diagnostic usefulness.

The technique followed in the cases to be cited was as follows: The skin area over the body of the sternum at the level of the third interspace was cleaned with iodine and alcohol, and then anesthetized with 2 per cent novocain down to and including the periosteum. The needle used for puncturing the sternum was No. 18 gauge lumbar puncture needle with stylet in place, shortened to approximately 5 cm. according to the method suggested by Young and Osgood.¹² It was introduced at an angle of approximately 70°, and with a steady downward and somewhat rotatory pressure the anterior cortex was readily penetrated. After withdrawing the stylet, a tightly fitting 10 c.c. syringe was used to aspirate approximately 4 c.c. of sanguineous "marrow juice." This was transferred to media and treated in a manner similar to blood culture material. Most satisfactory growth was obtained on media containing selenite F.

Three patients with typhoid fever admitted to the University Hospital during August, September, and October, 1939, afforded an opportunity to observe the value of this procedure. Sternal marrow cultures were obtained once in each instance, i.e., on the fourteenth, nineteenth, and twenty-first days of the illness, respectively. Data concerning other bacteriologic and serologic studies in these cases, together with brief abstracts of the case histories, are cited.

CASE 1.—W. E. (Chart No. 27343), a white male, aged 14 years, was admitted to the Medical Service at the University Hospital on September 12, 1939, complaining of weakness, loss of appetite, and fever of two weeks' duration. Further questioning elicited complaints of more or less constant headache, marked constipation, and pain in the abdomen. The patient told of swimming in the harbor on several occasions some days prior to onset of illness. Examination revealed a very toxic, somewhat lethargic boy, with an admission temperature of 101.4° F. and a pulse rate of 88 per minute. Scattered small erythematous papules, which faded on pressure, were observed over the chest, back, and abdomen. A few sonorous and wheezing râles were heard over the bases of both lungs. The tip of the spleen was palpable 4 cm. below the left costal margin and the edge of the liver was likewise felt.

Aside from cellulitis of the right thigh which subsided uneventfully, the patient's course was entirely typical for typhoid fever, with persistence of hyperpyrexia for three weeks and gradual return to normal by lysis. Bone marrow culture on the twenty-first day of the illness was positive, and a simultaneous blood culture was negative. Agglutination

titer, with both O and H antigens, never rose above 1:160. In this instance, the diagnosis having been established by prior positive cultures, bone marrow culture was merely of academic interest.

TABLE I
CASE 1. SEROLOGIC AND BACTERIOLOGIC EXAMINATIONS

DATE	DAY OF DISEASE	BLOOD CULTURE	URINE CULTURE	STOOL CULTURE	BONE MARROW CULTURE	AGGLUTINATIONS
9/13/39	16th	+				1:160 +
9/14/39	17th	-		+		
9/16/39	19th	-				
9/18/39	21st	-			+	
9/19/39	22nd		+			
9/21/39	24th	-				1:80 ++
9/29/39	31st			-		
10/ 1/39	33rd		-			
10/ 8/39	40th					1:80 ++
10/ 9/39	41st		-	-		
10/10/39	42nd		+			
10/11/39	43rd		+			

CASE 2.—A. F. (Chart No. 26969), a white female, aged 11 years, was admitted to the University Hospital on August 26, 1939, complaining of malaise and feverishness of thirteen days' duration. Illness was insidious in onset, with progressive drowsiness, fever, frequent headaches, and anorexia. Examination revealed a well-nourished, well-developed, somewhat toxic young girl, whose admission temperature was 105° F. and whose pulse rate was 110 per minute. Numerous "rose spots" were seen on the back, arms, and abdomen. A harsh mitral systolic murmur was heard. The spleen was not palpable.

TABLE II
CASE 2

DATE	DAY OF DISEASE	BLOOD CULTURE	BONE MARROW CULTURE	STOOL CULTURE	URINE CULTURE	AGGLUTININS (POSITIVE UP TO)
8/26/39	14th	-				
8/28/39	16th			-	-	1:320 +
8/29/39	17th			-	-	
8/30/39	18th	-		-		
8/31/39	19th		+	-	+	
9/ 2/39	21st	-		-		
9/ 5/39	24th					1:320 ++
9/ 6/39	25th	-		-	-	
9/ 8/39	27th			+		
9/11/39	30th	+		-		
9/12/39	31st	Relapse				
9/13/39	32nd	+				1:320 +
9/18/39	37th			-	-	1:160 +
9/21/39	40th	-		-		
9/26/39	45th	-		-		
9/28/39	47th			-	-	1:160 +
10/ 2/39	48th			-	-	
10/ 3/39	51st			-		
10/ 9/39	52nd	-				
10/16/39	58th	-		-		1:320 +++
10/19/39	65th	-		-		1:320 +
10/20/39	68th	-				
	69th					1:320 ++

All bacteriologic examinations remained negative until August 31, when both bone marrow and urine cultures were found to contain *E. typhosa*. The agglutinin titer, however, two days after admission was positive up to a dilution of 1:320. Thus, in this instance, the

first definite bacteriologic evidence of typhoid fever was a positive marrow culture on the nineteenth day of illness. The patient's course in the hospital was marked by subsidence of fever within a week after admission, only to be followed a few days later by recurrence of hyperpyrexia, associated this time with several positive blood cultures. Recovery, however, was essentially uneventful, and the patient was discharged on October 22, 1939.

CASE 3.—D. R. (Chart No. 27929), a white female, aged 14 years, was admitted to the University Hospital on October 7, 1939, complaining of malaise, fever, and listlessness which began on September 28. The illness was characterized by sudden onset with a chill followed by elevation of temperature to 104° F. On succeeding days there was recurrence of chills. On October 1, following therapy with quinine, there occurred a generalized wheallike eruption which lasted for twenty-four hours. Because of a known case of typhoid in the vicinity and continued hyperpyrexia, hospitalization was advised. Examination on admission showed a well-developed, well-nourished white female of 14 years, lying quietly in bed. The temperature was 100° F. and the pulse rate was 80 per minute. The remainder of the physical examination at this time was essentially negative. The laboratory studies were likewise noncontributory. Agglutination studies for typhoid were positive only up to titer of 1:80. Four days after admission, i.e., on the fourteenth day of illness, bone marrow culture was reported positive for *E. typhosa*. This was the first definite bacteriologic evidence of the etiology of the hyperpyrexia. It will be observed that blood cultures remained persistently negative in this case.

TABLE III

CASE 3

DATE	DAY OF DISEASE	BLOOD CULTURE	BONE MARROW CULTURE	STOOL CULTURE	URINE CULTURE	AGGLUTINATION
10/ 7/39	10th	—				1:80 +
10/ 9/39	12th	—		—	—	1:80 +
10/11/39	14th		+			1:160 ++
10/12/39	15th				—	
10/13/39	16th				—	1:80 +
10/16/39	19th	—		+	—	
10/19/39	22nd	—		—	—	1:80 +
10/20/39	23rd				—	
10/21/39	24th	—		+	—	
10/24/39	27th			—	—	
10/25/39	28th	—		—	—	
10/31/39	34th			—	—	1:80 +
11/ 1/39	35th				—	
11/ 3/39	37th			—	—	

COMMENT

The rationale for the bacteriologic examination of bone marrow exists in the fact that in this situation one may possibly secure capillary (arterial) blood as well as material rich in phagocytic reticulo-endothelial cells. These factors together are believed to offer greater probability of obtaining positive results than culture of venous blood taken at a distance from the locus of infection. In typhoid fever the growth and proliferation of organisms in the marrow has been known for many years. However, it remained for the development of a relatively simple technique, i.e., sternal puncture, to stimulate the clinical exploitation of this source of bacteria. The limited number of our cases does not permit at this time any broad generalizations as to the comparative value of this diagnostic procedure. However, our experience, as well as a consideration of the recent literature, seems to suggest that culture of the marrow offers a reliable and constant source of organisms during the acute disease from the earliest

days of the illness to the end of the febrile period. The procedure is, therefore, of value in early diagnosis, but appears to have its greatest usefulness at a time, roughly between the tenth and fifteenth days of the disease, when current diagnostic laboratory procedures are positive in only about 70 per cent of the instances. The further value of this technique, moreover, appears to us to lie in its possible extension to other types of infection associated at one time or another with bactericemia.

SUMMARY

1. Bacteriologic culture of sternal marrow obtained by needle puncture on the fourteenth, nineteenth, and twenty-first days of the illness in three cases of typhoid fever was uniformly positive.

2. Further work seems indicated to establish the value of this procedure in typhoid fever and other infections.

REFERENCES

1. Longcope, W. T.: Eine Studie über das Knochenmark bei Typhus und Anderen Akuten Infektionskrankheiten, *Centralbl. f. Bakt. Abt. 1*, 37: Ref. 23: 112, 1905.
2. Ludke, H.: Ueber den latenten Mikrobismus der Typhusbazillen, *München med. Wchnschr.* 56: 57, 1909.
3. Gay, F. P.: *Typhoid Fever*, New York, 1918, Macmillan Co.
4. Ghedini, G.: Per la patogenesi e per la diagnosi delle malattie del sangue e degli organi ematopoietici, puntura esplorativa del midollo osseo, *Clin. med. ital.* 47: 724, 1908.
5. Zadek, I.: Blut- und Knochenmarkbefunde am Lebenden bei kryptogenetischer perniziöser Anämie, insbesondere im Stadium der Remission, *Ztschr. f. klin. Med.* 95: 66, 1922.
6. Peabody, F. W.: The Pathology of the Bone Marrow in Pernicious Anemia, *Am. J. Path.* 3: 179, 1927.
7. Seyfarth, C.: Die Sternuntrepanation, eine einfache method zur diagnostischen Entnahme von Knochenmark bei Lebenden, *Deutsche med. Wchnschr.* 49: 180, 1923.
8. Arinkin, M. I.: Die Intravitale Untersuchungsmethodik des Knochenmarks, *Folia haemat.* 38: 233, 1929.
9. Chung, H. L.: Sternal Puncture Technic and Its Clinical Value, With Especial Reference to Its Usefulness in the Diagnosis of *Kala-azar*, *Chinese M. J.* 54: 397, 1938.
10. Osgood, E. E.: Phagocytosis of Malaria Parasites by Neutrophil Leukocytes of Marrow, *Proc. Soc. Exper. Biol. & Med.* 33: 219, 1935.
11. Bock, H. E.: Ueber den Wert der Bakteriologischen Sternalmarkuntersuchung, *Klin. Wchnschr.* 18: 162, 1939.
12. Signorelli, S.: Quoted by Baserga and Barbagallo.
13. Baserga, A., and Barbagallo, G.: Die Sternalpunktion als bakteriologische Hilfsmethode, *Med. Klin.* 34: 178, 1938.
14. Gerbasi, M.: Ricerche sulla patogenesi e sulla diagnosi della febbre tifoide nei bambini, *Pediatrics* 32: 113, 1925.
15. Debré, R., Lamy, M., Sée, G., and Mallarme, J.: L'exploration de la moelle osseuse (la myelographie et la medullo culture), *Presse méd.* 44: 1853, 1936.
16. Storti, E., and Filippi, C.: Étude morphologique et bactériologique de la moelle osseuse dans la fièvre typhoïde, *Sang* 11: 440, 1937.
17. Young, R. N., and Osgood, E. E.: Sternal Marrow Aspirated During Life; Cytology in Health and in Disease, *Arch. Int. Med.* 55: 186, 1935.

IMMUNOLOGIC TEST FOR HUMAN BLOOD AND HUMAN PROTEIN IN STOOLS*

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IT IS well known that protein is generally absent in human stools but that there are occasional cases in which presence of protein may be shown by chemical methods.

It is of the greatest importance to determine whether such protein found in the stool is derived from food not sufficiently digested or from the human body because of the vastly different significance of these findings. Hence it seems of practical value to have a readily available method by means of which protein in the stool can be easily identified as being derived from the human body.

Since we have no special chemical test for human blood protein, we must make use of immunochemistry. Injecting human serum into rabbits, the antiserum developed after a certain length of time will react in a specific way, giving a specific precipitate with human serum protein.

To prepare this antiserum (precipitin), human serum free of hemoglobin is diluted ten times with saline and after being slightly acidified (to pH 6.5) is absorbed on the same volume of aluminum cream following the procedure of Hektoen and Welker. After centrifuging until no protein is left in the supernatant fluid, the fluid is discarded and the remaining precipitate is mixed with saline so that 1 c.c. of this emulsion corresponds to 3 c.c. of diluted or 0.3 c.c. of original serum. Twenty cubic centimeters of this emulsion are injected at different places in the muscles of the hind legs of a rabbit forming a few deposits from which the protein may be absorbed into the blood. The antibody has usually reached a maximum at the end of two weeks. After a test has shown a titer of 1:100,000 with human serum, the rabbit is bled to death and the serum is ready for the test. It is kept in the refrigerator for further use.

For the stool precipitin test the stool is well mixed so as to secure a thorough distribution of the protein in the stool. As it is rather difficult to secure a completely uniform stool mixture, several portions of the well-mixed stool may have to be examined. The stool specimen is thoroughly mixed with five parts of 0.9 per cent sodium chloride solution. The stool suspension is centrifuged, the supernatant fluid is filtered through good filter paper, and the filtrate is returned to the filter until the filtrate appears clear. Clearing the filtrate by use of adsorbents, such as kaolin or Fuller's earth, or by using a Berkefeld filter is not admissible, as by such procedure protein would be removed. As soon as the filtrate is clear it is ready for the test.

For the test we employ very small test tubes, 3 mm. in diameter and 1 inch high. Pipettes with very fine points are made from glass tubing. With one of

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these pipettes we place in the tube a small amount of stool filtrate so that the tube is about one-third filled. With another fine pipette we lay about the same amount of antiserum below the stool filtrate, taking care that the point of the pipette goes through to the bottom of the tube. If human protein is present, a white ring—the specific precipitate—will develop at the border of the two layers. In case the protein content is rather high, the ring will appear immediately. If no ring appears within one hour, the test is negative.

The reaction is specific for human protein and is negative for protein from food or bacterial bodies. Hence if positive with stool extract, it is specific for colitis, exudates, or bleeding from the colon.

For differentiating between exudate and bleeding the absence or presence of human blood corpuscles must be determined. This can also be done immunologically, testing by the precipitation method for human hemoglobin.

To prepare an antiserum for human globin, rabbits are injected with a solution of human hemoglobin. This is prepared by hemolyzing thoroughly washed human corpuscles by adding slowly a small amount of ether (10 to 12 drops to 100 c.c.), shaking two to three minutes and repeating this procedure a few times until the corpuscles are completely hemolyzed. This solution is mixed with three parts of aluminum cream and centrifuged. The clear red solution devoid of serum protein and containing the hemoglobin may be used as an antigen for producing a precipitin specific for human globin. Injecting a rabbit every third day with increasing amounts of this solution, 1 c.c., 2 c.c., 3 c.c., 5 c.c., 8 c.c., and 10 c.c., one week after the last injection the serum may give the precipitin test with a hemoglobin solution 1:100,000. The test is made in the same way as described before for proving the presence of human protein. Positive test of the stool extract with this antiserum demonstrates the presence of human globin and thus of hemoglobin, and shows, together with the positive test for human blood protein, that bleeding occurred some place in the intestine.

CONCLUSIONS

1. By precipitin reaction, using as antibody a specific human serum-rabbit antiserum, the presence of human blood protein may be detected in human stools.
2. Using as antiserum a human hemoglobin-rabbit antiserum, the presence of human corpuscles or derivatives thereof can be demonstrated.
3. Positive precipitin reaction of stool extract with both serum antiserum and globin antiserum is characteristic for bleeding, whereas precipitin reaction with serum antiserum and negative test with globin antiserum will indicate an exudate from the bowels as produced by an exudative process.
4. Testing with dilutions of the feces extract will give a rough quantitative estimation.

ANTHRAX IN SHAVING BRUSHES*

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THE finding of anthrax spores in toothbrushes, hairbrushes, or shaving brushes occurs infrequently. Smyth (1939) reviews a twenty-year survey of anthrax in the United States.¹ In his tabulation of "Cases and Deaths by Source of Infection" appear 2,044 cases and 136 deaths where the source of infection is other than "transportation," "agricultural," or "professional." Such definite commercial commodity data have been adapted to Tables I and II.

TABLE I

ANTHRAX CASES AND DEATHS HAVING DEFINITE TRADE COMMODITY SOURCES OF INFECTION 1929-1938 INCLUSIVE

COMMERCIAL COMMODITY	CASES	DEATHS	TOTAL CASES (PER CENT)	TOTAL DEATHS (PER CENT)
Hides and skins	1,113	58	54+	43-
Wool	565	34	28-	25
Hairbrushes	207	18	10+	13+
Shaving brushes	159	26	8-	19+
Total	2,044	136 (7 -%)		

+ signifies slightly more than the whole number given.

- signifies slightly less than the whole number given.

TABLE II

COMPARISON OF COMMERCIAL COMMODITY SOURCES OF INFECTION WITH DEATHS REPORTED

COMMERCIAL COMMODITY	CASES	DEATHS	DEATHS TO CASES
Hides and skins	1,113	58	5+
Wool	565	34	6+
Hairbrushes	207	18	9-
Shaving brushes	159	26	16+

Tables I and II emphasize that, although shaving brushes represent slightly less than 8 per cent of this grouping of industrial cases, yet the highest percentages of deaths occur in the shaving brush source group. The higher vascularity of tissue exposed and the minute nicking of the epidermis during shaving both favor the introduction of the organism into a suitable environment for propagation.[†]

The isolation and identification of *Bacillus anthracis* are simple laboratory tasks. However, in the examination of suspected shaving and similar brush material, a "hunch" in our own experience seems worthy of reporting, as evidence of extending sampling a bit further than in the examination of the hair or bristles of the brush proper.

Smyth contends, "The latest outbreak of shaving brush cases is due to shipments of cheap brushes made from 'sterilized' horsehair, imported from Japan." We were interested in the possible presence of such brushes in our community.

*From the Public Health Laboratories, Los Angeles County Health Department, Los Angeles.²

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[†]Concerning shaving brushes, Smyth states, "They have a fatality rate of from 50 per cent to 60 per cent."

Early in 1939 our sanitary inspectors were asked to find out if "Imperial, Sterilized Japan, 332" shaving brushes were in stock for sale in our local stores. Only one such brush was found. It was purchased in a 5 and 10c store, by inspector W. C. Weymouth of our department. The brush was sealed well in cellophane, and was an attractive appearing piece of merchandise. Much to our surprise, this single sample yielded anthrax organisms by direct culture and from guinea pig inoculations. This brush was the only one of this brand found still unsold.

Radio and press requests were made in order to contact purchasers of this brand of brush. Nine used brushes were submitted to us as a result, one being received from a mountain town about 350 miles distant from Los Angeles.

These brushes had been in active use by the owners; none yielded anthrax organisms from the brush portion. Only one of the owners felt that he had experienced an "infection." This man stated that he had bought the brush about a year before. He "now recalled" that he had probably experienced an anthrax infection following the initial use of the brush. Our interested inquiry as to what kind of symptoms he experienced brought the reply of "no sores, but a peculiar feeling under the skin." We asked "What sort of peculiar feeling?" The answer was "Oh! like ants crawling around under the skin, you know, ant tracks!" Thus is our terminology sometimes misinterpreted by the layman.

We did not expect to recover anthrax from these used brushes, but examination of the merchandise led to an attempted innovation in such an examination.

The handles of hard wood were broken open, and the resinous brittle gum was crushed, liberating the ends of the hairs from their protective seal. The handle end of the hair was then subjected to culture and easy procedures. One of the nine yielded a luxuriant growth, and gave typical post-mortem evidence in a cavity; the heart-blood culture yielded pure colonies of anthrax.

Inasmuch as this brush had been purchased either late in 1937 or early in 1938 and had been in constant use up to March, 1939, it is interesting to conjecture on whether or not the actual brush tuft portion had been effectively sterilized after the handle and hair had been put together, or had the user simply been fortunate is not contracting anthrax during the period of first use, before the spores were removed as a result of the daily washing out of the brush?

SUMMARY

Breaking open the handles of bristle or hairbrushes, removal of the handle binder from the hairs, and subsection of this portion of the hair to cultural and animal procedures, for the demonstration of anthrax, yielded a positive result in one of nine used shaving brushes of Japanese manufacture.

REFERENCES

1. A Twenty Year Survey of Anthrax in the United States, Sixth Report of the Committee on Anthrax, Industrial Hygiene Section, A. P. H. A., October 18, 1939, Dr. Henry Field Smyth, Chairman.
2. Release for publication approved by Dr. J. L. Pomeroy, Los Angeles County Health Officer, March 25, 1940.

LABORATORY STUDIES IN THE FALCIPARUM MALARIA OF DRUG ADDICTS*

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THE literature contains many clinical pathologic studies concerning the disease of malaria. The recorded observations deal with the various types of malaria resulting from natural mosquito infection, and more recently, from the malaria induced for the therapy of nervous system syphilis. Except for the notes on "Laboratory findings" contained in Helpern's paper,¹ we have been unable to find any clinical pathologic studies of the falciparum malaria of drug addicts resulting from the common use of hypodermic syringes for intravenous heroin administration.

Falciparum malaria in drug addicts is now a well-established and recognized entity in New York City and probably also occurs in considerable numbers in other large metropolitan areas. Studies on the epidemiology and clinical aspects of the disease are reported elsewhere.²

During the past six years we have had the opportunity of observing well over two hundred drug addicts suffering from falciparum malaria contracted as a result of the common use of hypodermic syringes for intravenous heroin administration. All clinical varieties of falciparum malaria were encountered, including fatal cerebral, gastrointestinal, and hemoglobinuric syndromes. In many cases detailed laboratory studies were carried out. It is the object of this paper to present the findings of these studies.

MATERIAL AND METHODS

The patients studied in this series were drug addicts in whom a proved diagnosis of falciparum malaria was made. They were hospitalized, observed, and treated on the wards of the Third Medical Division and the Medical Service of the Psychiatric Division of Bellevue Hospital, New York.

Blood counting was done with the aid of certified blood pipettes. Hemoglobin was determined by an acid hematin method using a Klett-Newcomer discolorimeter. The mean corpuscular volume was determined by the Wintrob method.

The nonprotein nitrogen was done by the micro-Kjeldahl method, and blood sugar by the Folin-Wu method; serum bilirubin by the Ernst-Förster method and plasma proteins by the tyrosine method. The protein in the spinal fluid was precipitated and determined by the micro-Kjeldahl method.

Urobilinogen was determined by the dilution method of Wallace and Diamond using Ehrlich's reagent (paradimethylaminobenzaldehyde).

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TABLE I
HEMATOLOGIC DATA ON ADMISSION

Red Blood Cells		
R.B.C. millions per c.mm.		Cases
1-1.9		2
2-2.9		19
3-3.9		19
4-4.9		27
Over 5		7
Hemoglobin		
Grams per 100 c.c.		Cases
Less than 5		2
5-10		32
10.1-15		36
Over 15		3
Mean Corpuscular Volume		
Cubic microns		Cases
Less than 80		6
80-94		9
Above 94		9
White Blood Cells		
W.B.C. thousands per c.mm.		Cases
Less than 5		9
5-10		35
Over 10		31
Lowest count	3.25	
Highest count	46.00	
Monocytosis (above 15 per cent)		Cases
Present*		12
Absent		63

*Ingested pigment frequently found.

HEMATOLOGIC OBSERVATIONS

Red Blood Cells, Hemoglobin, and Mean Corpuscular Volume.—In the hematologic tables one sees that in about half the individuals studied, considerable anemia existed. In 28 per cent the red blood cell count ranged between 1 and 2.9 million cells per cubic millimeter and in 46 per cent the hemoglobin was reduced below 10 Gm. per 100 c.c. On the other hand, in many individuals, despite severe malarial infection, little or no anemia existed.

The mean corpuscular volume was determined in 24 patients. The results obtained in 15 showed that the size of the red blood cells is in the normal range. In 9 patients, however, there was definite macrocytosis. Since there is no corpuscular enlargement associated with the development of *P. falciparum*, another explanation must be sought for the macrocytosis. Certainly these cases are complicated by dietary insufficiency and possibly by liver damage. These factors may account for the phenomenon of macrocytic anemia. It is of interest to note that Fairley and Bromfield,³ in studying the average diameter of the red blood cells in 14 patients with *P. falciparum* infection (not drug addicts) by the balometer, found them all within the normal range. He doubts the existence of true megalocytic anemia in uncomplicated cases. Certainly the disease in the drug addict is not simple, uncomplicated malaria. Prolonged dietary inadequacy associated with chronic drug addiction, in many cases alcoholism and intrinsic

TABLE II
BLOOD CHEMICAL DATA ON ADMISSION

Nonprotein nitrogen										
Milligrams per cent								Cases		
20-35								41		
36-50								13		
51-100								8		
Blood sugar										
Below 100								23		
101-150								23		
151-200								6		
Serum bilirubin										
0.5-1.0								17		
1.1-2.0								15		
Above 2.0								18		
Van den Bergh (direct reaction)										
Result								Cases		
Immediate								18		
Delayed								16		
Negative								13		
Plasma Proteins Studied in 19 Cases										
Serum albumin*										
Gm. per 100 c.c.	3.0	3.7	3.4	2.6	3.4	3.5	2.7	4.1	2.4	
Serum globulin	3.5	3.4	3.1	3.2	2.8	2.8	2.0	3.4	3.5	
Total	6.5	7.1	6.5	5.8	6.2	6.3	4.7	7.5	5.9	
Serum albumin	2.4	2.1	3.0	4.0	2.3	2.4	2.8	2.2	5.3	2.5
Serum globulin	2.4	2.2	1.6	2.5	2.2	2.1	2.7	1.6	2.2	2.3
Total	4.8	4.3	4.6	6.5	4.5	4.5	5.5	3.8	7.5	5.3
Analysis of Plasma Proteins										
Serum albumin										
Grams per 100 c.c.								Cases		
2.0-3.0								12		
3.1-3.7								4		
4 or over								3		
Serum globulin										
Grams per 100 c.c.								Cases		
1.5-2.8								13		
2.9-3.5								6		
Albumin-Globulin ratio										
Normal								3		
Reduced or inverted								16		
*Normal values Albumin 4.0-5.3.										
Globulin 1.8-2.7.										
Total 6.5-7.9.										

or malarial liver disease, may constitute factors in the mechanism of the anemia seen in these addicts, in addition to that directly dependent on the malarial infection itself.

White Blood Cell Count and Monocytosis.—From a diagnostic point of view, the white blood cell count and the differential formula, especially with reference to the number of monocytes, is seen to be of little value. In our series the white blood cell count was found elevated in 31 cases, normal in 35 cases, and less than normal in 9 cases. Monocytosis was seen only in 12 of 75 cases in which a differential count was done. Unfortunately this finding, so much stressed in texts, would appear to be of little value as a diagnostic aid. The presence of ingested pigment, however, is strong presumptive evidence of malarial infection.

BLOOD CHEMICAL OBSERVATIONS

Nonprotein Nitrogen.—The blood urea is frequently elevated in falciparum malaria. The nonprotein nitrogen was determined in 62 patients. Values between 51 and 100 milligrams per cent were found in 8. In two of these patients kidney disease (acute or chronic glomerulonephritis) was known to exist, but in the remaining 6 the elevation was felt to be associated with the malarial process and was dependent on one or more of the following: dehydration, vomiting, anoxemia, anemia, and hemoconcentration. In the cases in which recovery followed treatment, but no kidney disease existed, the values returned to normal very quickly.

Blood Sugar.—The blood sugar was found elevated above 150 milligrams per cent in 6 patients. In these one or more of the following was present: severe vomiting, hemoconcentration, high fever, anemia, or impaired liver function. We feel that one or more of these factors in our series may explain temporary derangement of the carbohydrate regulatory mechanism.

Serum Bilirubin.—Values of serum bilirubin up to 1.0 milligram per cent were considered normal. Sixty-six per cent of the persons studied exhibited hyperbilirubinemia. In the cases in this series, whenever the height of the serum bilirubin was considerable, the van den Bergh reaction was of the direct immediate type. The higher levels were associated also with moderate anemia and clinical icterus, as well as with urobilinuria.

Plasma Proteins.—The total proteins were reduced below 6.0 Gm. per cent in 11 of 19 individuals whose plasma proteins were studied. In the main this reduction, as well as a lowering or inversion of the albumin-globulin ratio, was due to the depression in the value for serum albumin. The latter values were considerably below normal in at least 16 of the total number studied. Values for the serum globulin, on the other hand, were essentially normal, although definitely increased in 6 patients.

SPINAL FLUID

It is surprising that in the face of the severe involvement of the brain, which occurred so often, relatively few changes were observed in the composition of the spinal fluid.

SEROLOGIC DATA

The Wassermann reaction was strongly positive (4 plus) in 15 patients, weakly positive in 2 and doubtful in 3. Even though one may concede a greater incidence of syphilis among drug addicts, one would hardly expect such a high percentage of syphilis if the positive Wassermann is interpreted as evidence of syphilitic infection. In 8 persons in whom the Wassermann was 4 plus at the height of the malaria, the reaction was negative after recovery. Two weakly positive and two doubtful reactions likewise became negative after recovery from active malaria. That the Wassermann reaction in malaria may be positive in the absence of syphilis is well appreciated in the tropics and elsewhere⁴ and is re-emphasized in this series.

Henry's Serum Flocculation Test for Malaria (Chorine Modification).⁵—This test was positive in the 25 patients in whom it was carried out. We performed it only as a matter of academic interest, since in all cases the diagnosis

TABLE III
SPINAL FLUID DATA

Clear	23
Grossly bloody (not technical) not xanthochromic	2
Xanthochromic	2
White blood cells (more than 10 per c.mm.)	2
Globulin present	10
Globulin absent	15
Reducing substances present	25
Reducing substances absent	0
Wassermann negative	25
Wassermann positive	0
Colloidal gold curve normal	25
Colloidal gold curve abnormal	0
Chemical—Quantitative	
Protein—mg. %	Cases
5-15	6
16-30	1
Sugar	Cases
40-60	3
61-90	5
Chlorides	Cases
Below 700	5
700-750	4
Above 800	1

TABLE IV
SEROLOGIC DATA

Blood Wassermann Reaction	
Result	Cases
Strongly positive	15
Weakly positive	2
Doubtful	3
Negative	46
Henry's Serum Flocculation Test for Malaria (Chorine Modification)	
Performed in 25 cases	
Dilution	Cases
1:200	8
1:450	10
1:1,000	5
1:1,200	2
Positive serum is one showing flocculation in dilution of 1:200 or higher.	

TABLE V

Urinary Findings	
Examination	Positive
Albumin	27
Acetone	5
Reducing substance	5
Bile	7
Casts	12
Red blood cells in excess	10
Methemoglobin	2
Urobilinogen	
Dilution	Cases
1:20	17
1:40	13
1:80	13
1:160	12
1:320	6
1:1,000 and above	2

was established previously by an examination of a stained blood smear. The test may be of value if plasmodia cannot be found, as in latent or chronic or unusual forms of malaria.

URINARY FINDINGS

The urinary findings were not striking. Albumin, acetone, bile, reducing substance, and methemoglobin were present in small amounts, and red blood cells and casts in small numbers. Any or all of the findings enumerated in Table V may be explained by the malarial infection per se. On the other hand, cerebral involvement, vomiting, starvation, dehydration, diarrhea, ingestion of other drugs, hemorrhage, and intravascular hemolysis, which operated in many cases in this series, might equally serve to explain the urinary findings here reported. The output of urinary urobilinogen is also represented in Table V. It is of note that in 46 persons values of 1:40 dilution or higher were found. Hemoglobinuria appeared in the two patients with clinical blackwater fever.

SUMMARY

The clinical pathology of falciparum malaria of drug addicts is presented. None of the laboratory findings, other than direct blood smear examination, is diagnostic of the disease. On the whole, the laboratory observations are those seen in natural and induced malaria. The outstanding findings are:

1. Anemia, which may be macrocytic.
2. Hyperbilirubinemia with an altered van den Bergh reaction.
3. Hypoproteinemia, due on the whole to depression in the value of the serum albumin and resulting frequently in reduction or inversion of the albumin-globulin ratio. The serum globulin may be considerably elevated.
4. Relatively high incidence of positive Wassermann reactions.
5. Positive Henry's flocculation test.
6. Increased urinary output of urobilinogen.

REFERENCES

1. Helsen, M.: Epidemic of Fatal Estivo-autumnal Malaria Among Drug Addicts in New York City Transmitted by Common Use of Hypodermic Syringe, *Am. J. Surg.* 26: 111, 1934.
- 2 (a) Most, H.: Falciparum Malaria Among Drug Addicts. *Epidemiologic Studies*, *Am. J. Pub. Health* 30: 403, 1940.
- (b) Most, H.: Falciparum Malaria Among Drug Addicts. *Clinical Aspects*, *Am. J. Trop. Med.* 20: 1940.
3. Fairley, N. H., and Bromfield, R. J.: Laboratory Studies in Malaria and Blackwater Fever, Part I. Malaria, *Tr. Roy. Soc. Trop. Med. & Hyg.* 27: 289, 1933.
4. Kitchen, S. F., Webb, E. L., and Kupper, W. H.: Influence of Malarial Infections on the Wassermann and Kahn Reactions, *J. A. M. A.* 112: 1443, 1939.
5. Chorine, V.: Les réactions sérologiques dues aux euglobulines, *Ann. Inst. Pasteur* 58: 78, 1937.

A COTTON-PLUG WRAPPING MACHINE FOR BACTERIOLOGIC CULTURE TUBES AND BOTTLES

L. L. KEMPE AND H. R. SHIPMAN,* MINNEAPOLIS, MINN.

IN MANY bacteriologic laboratories large numbers of cotton plugs are required for use as stoppers in culture tubes, flasks, and bottles. The demand for such plugs is particularly large where water samples are tested by the Most Probable Number method. It has been found that considerable experience is necessary before an individual becomes sufficiently skilled to make good plugs by hand methods at even a reasonable rate. It seemed probable to us that a simple machine could be constructed that would produce better plugs at a faster rate. Such a machine was put together from miscellaneous materials at hand in our laboratory. It consists of the following parts:

1. A one-thirtieth horse power, induction wound, electric motor.
2. A mandrel fashioned from a sixteen-penny finishing nail which was filed to a triangularly tapered point, and then polished with fine sandpaper.
3. A gear-operated speed reducer connected directly with the motor that turned the mandrel approximately 170 r.p.m.
4. A foot switch of the instantaneous action, circuit-breaker type.

The motor and speed reducer are one unit which was originally designed to revolve a brush used in washing test tubes. The chuck, in which the test tube brush was formerly placed, is used to hold the mandrel. In order to adapt this machine for use as a test tube plug wrapper, it was mounted firmly on a work bench. This was accomplished with two "C" clamps purchased at a hardware store.

Fashioning of the mandrel was relatively simple. A sixteen-penny nail was placed in a vise and the head was filed off. The ends were then reversed in the vise and the nail was filed to a triangularly tapered point which was about three-fourths as long as the plug desired. It is extremely important to polish the mandrel because file marks or other irregularities will catch cotton fibers and make the plug difficult to remove. The polishing operation should be carried out with lengthwise strokes, using very fine sandpaper. The finished mandrel mounted in the chuck on the electric motor is shown in Fig. 1.

A sensitive, instantaneous action foot switch is necessary to provide positive control of the motor. Our first switch was made from a doorbell push button mounted between two boards. This has proved satisfactory. A similar switch made for photograph enlarging machines could be adapted for use with this machine. Such a switch can be purchased at most photographic supply stores.

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The operation technique depends somewhat on the type of plug desired and on the operator. We have found the following system to be simple and fast:

1. A roll of cotton is separated into layers of suitable thickness. For test tube plugs an average roll is separated into three thicknesses, each of which is cut into four strips (one strip will make several plugs).

2. One end of a cotton strip is placed over the mandrel's tapered end, as is shown in Fig. 2. The machine is started by depressing the foot switch, and the cotton is fed onto the plug until sufficient material has accumulated. The plug is shaped between the thumb, index finger, and middle finger of the left hand.



Fig. 1.



Fig. 2.



Fig. 3.

Fig. 1.—View of chuck and mandrel showing manner in which mandrel is formed from a sixteen penny finishing nail.

Fig. 2.—View from the operator's position showing cotton being fed onto the mandrel. A foot switch (not shown) starts and stops the electric motor.

Fig. 3.—The finished plug is easily pulled from the mandrel when the motor is stopped.

3. At this point, with the machine still running, the strip is torn from the plug which is then finished with the fingers of the left hand. Varying pressure of the fingers will vary the tightness and hardness of the finished plugs. A finished plug is shown in Fig. 3.

4. The formed plug is now guided into a test tube, the motor is stopped, and the plugged tube is removed from the mandrel. If an extremely hard plug has been made, it may be necessary to twist the plug about one-quarter turn opposite to the direction of formation in order to detach the plug easily.

After almost one year of continuous operation the machine originally constructed is still in good operating condition. The only care it has received consisted of occasional cleaning and greasing.

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to clinical use. That of Kodieck⁶ employs p-aminoacetophenone and few enough operations to be an apparently satisfactory routine method.

The reliability and simplicity of the method to be described as tested by its application to approximately 250 blood specimens seemed to warrant a detailed report.

DETERMINATION

Apparatus and Reagents.—Any photoelectric colorimeter, or as was used in this laboratory, a Coleman "DM" photoelectric spectrophotometer.

10 per cent sodium tungstate and 2/3 N sulfuric acid.

2 per cent potassium dihydrogen phosphate, hydrochloric acid (36 per cent) and 10 N sodium hydroxide.

4 per cent aqueous cyanogen bromide, made fresh before each group of analyses. The solid material may be purchased from Eastman and keeps well in the icebox.

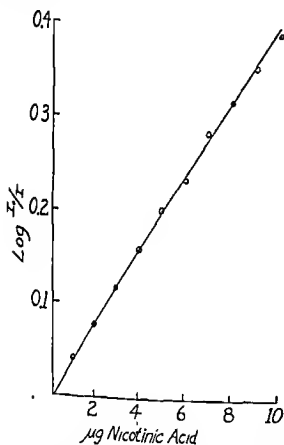


Fig. 1.

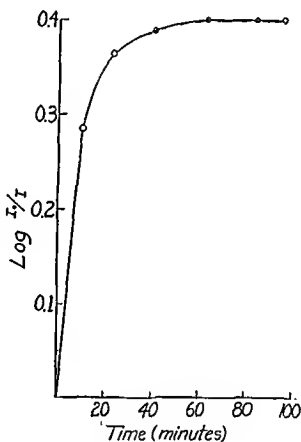


Fig. 2.

Fig. 1.—Linear relation between light absorption at $\lambda = 400 \text{ m}\mu$ and amount of nicotinic acid. Colors were determined sixty minutes after start of color development.

Fig. 2.—Rate of color development in the dark using $10 \mu\text{g}$ of nicotinic acid.

Saturated p-aminophenol ("metol"). Dissolve approximately 5 Gm. in 100 c.c. of water, shake for five minutes, and filter. This must be made fresh before each series of analyses.

Method.—Five cubic centimeters of oxalated blood is hemolyzed in 35 c.c. of water, followed by 5 c.c. of 10 per cent sodium tungstate and with shaking 5 c.c. of 2/3 N sulfuric acid. After standing for ten minutes, it is centrifuged for fifteen minutes at 2,500 r.p.m. to obtain the maximum amount of filtrate. The supernatant fluid may be filtered through a moistened filter paper to eliminate any floating precipitate, and 30 c.c. of the clear filtrate is measured into a 50 c.c.

centrifuge tube bearing a mark at 10 c.c. The tube is immersed in boiling water and a strong jet of air is blown on the surface for rapid evaporation to at least the 10 c.c. mark. (In practice 4 or 6 tubes may be treated simultaneously, requiring twenty to twenty-five minutes for the operation.) Water is added to the 10 c.c. mark, and 1.6 c.c. of concentrated (36 per cent) hydrochloric acid is added and mixed. The tube is immersed in a boiling water bath for fifteen minutes, cooled, and neutralized to pH 5.0 with 10 N sodium hydroxide (approximately 2 c.c.) and with 0.5 N sodium hydroxide as pH 5.0 is approached. This can be done with sufficient accuracy by removing small samples with a glass rod and testing on Hydrion A* indicator paper. The tube is replaced in the boiling water bath and with the air stream is evaporated to the point where salt begins to crystallize out (approximately 2 c.c.). It is centrifuged briefly and the liquid is poured off into a test tube marked at 6.0 c.c. Two successive small additions of water are added, washing down the walls. After centrifugation the water is poured into the test tube to the 6.0 c.c. mark and the contents are mixed. This tube is labeled A, from which 2.0 c.c. portions are removed to tubes B and C. Tubes A and B each receive 2.5 c.c. of 2 per cent acid phosphate, and tube C receives 2.5 c.c. of 2 per cent acid phosphate containing exactly 4 μ g of nicotinic acid. The tubes are placed in a water bath maintained at 75° to 80° C. Tube A receives 0.5 c.c. of water, tubes B and C, 0.5 c.c. of 4 per cent cyanogen bromide. After mixing they are allowed to remain for five minutes, cooled, and 5.0 c.c. of saturated p-aminophenol are added to each. The tubes are inverted for mixing and are placed in a dark closet for one hour, at which time they are ready for photoelectric measurement of the yellow color produced.

If the spectrophotometer is used, the contents of tube A are poured into the "solvent" tube, then the transmission of B and C successively measured at wave length $\lambda = 400 \text{ m}\mu$. In the case of the photoelectric colorimeter, a filter transmitting maximally at this wave length should be used. Since the color of C is due both to the unknown amount of nicotinic acid + 4 μ g of nicotinic acid (reacting under identical conditions as the unknown), then the amount of nicotinic acid in the unknown can be expressed as:

$$U = \frac{\log I_0/I_B}{\log I_0/I_C - \log I_0/I_B} \times 4 \mu\text{g}$$

and in milligrams per cent of total nicotinic acid in the blood, 0.1 U.

In the case of the photoelectric colorimeters, the "zero" reading must be set with tube A, then the relations above are similar except that the log of the galvanometer readings are utilized.

The use of tube C is generally necessary in nicotinic acid analysis since salt concentration differences, pH, etc., may affect the amount of color obtained during the coupling reaction. In the case of blood analysis under these conditions it was found, however, that the same amount of color was regularly obtained from 4 μ g of nicotinic acid in the presence of the prepared blood filtrate as in pure solution. In practice, therefore, the C tube was in-

*Distributed by R. P. Cargille, 118 Liberty Street, New York, N. Y.

cluded only occasionally. With our instrument and cells 4 μg of nicotinic acid gave a log = 0.160 ± 0.004 . The useful range of the instrument employed includes values up to 1.8 mg. per cent (fasting level 0.34 to 0.45 mg. per cent). If higher values are expected, smaller samples should be used for analysis.

For nicotinic acid analysis in urine, 15 c.c. of the urine are mixed with 2.0 c.c. of 10 N sodium hydroxide and heated in a boiling water bath for thirty minutes. After cooling it is neutralized with concentrated hydrochloric acid to pH 5.0 and water is added to 20 c.c. Two cubic centimeters of this solution (one may expect from normal persons in the order of 5 μg per cubic centimeter of urine) or appropriate dilutions thereof are put into tubes A, B, and C, as described under Method.

In this case it is essential to determine for each urine or dilution, the "color yield" of a standard amount of nicotinic acid under the conditions used (difference between tubes B and C).

TABLE I

REPRODUCIBILITY OF BLOOD NICOTINIC ACID DETERMINATIONS AND RECOVERY EXPERIMENTS

SAMPLE	NICOTINIC ACID ADDED* (MG./100 C.C. BLOOD)	THEORETICAL (MG. %)	FOUND (MG. %)	RECOVERY (%)
1	--	--	0.36†	--
	--	--	0.34†	--
	0.40	0.75	0.71	-5.3
	0.60	0.95	0.92	-3.2
	0.80	1.15	1.18	+2.6
	1.00	1.35	1.34	-0.7
2	1.50	1.85	1.74	-6.0
	--	--	0.39†	--
	--	--	0.38†	--
	0.20	0.59	0.59	0.0
	0.50	0.89	0.85	-1.5
	0.90	1.29	1.31	+1.5
	1.50	1.89	1.86	-1.6

*In normal saline.

†Corrected for the dilution of the blood caused by adding saline.

The method here described is essentially a composite of several tested methods, designed for clinical use. The data collected in Figs. 1 and 2 and in Table I demonstrate that with the procedure described (a) a perfectly straight line relation was obtained between the log of the transmission at $\lambda = 400 \text{ m}\mu$, and the amount of nicotinic acid; (b) that in the development of the color the reaction is 95 per cent complete in forty minutes, and constant from fifty-five to ninety-five minutes standing in the dark; (c) that in duplicate analyses of a single specimen of blood, results are reproducible within 6 per cent; and (d) that in recovery experiments where the nicotinic acid (in normal saline) is added directly to the blood, never less than a 92 per cent recovery was obtained in the range 3.0 to 15.0 μg of nicotinic acid.

REFERENCES

1. Ritsert, K.: The Determination of Nicotinic Acid and Amide in Urine, Tissues, and Blood, *Klin. Wchnschr.* 18: 934, 1939.
2. Pearson, P. B.: The Nicotinic Acid Content of the Blood of Mammalia, *J. Biol. Chem.* 129: 491, 1939.

3. Bandier, E., and Hald, J.: A Colorimetric Reaction for the Quantitative Estimation of Nicotinic Acid, *Biochem. J.* 33: 264, 1939.
4. Harris, L. J., and Raymond, W. D.: Assessment of the Level of Nutrition. A Method for the Estimation of Nicotinic Acid in Urine, *Biochem. J.* 33: 2037, 1939.
5. Melnick, D., and Field, H., Jr.: Chemical Determination of Nicotinic Acid: Inhibitory Effect of Cyanogen Bromide Upon the Aniline Side Reactions, *J. Biol. Chem.* 135: 53, 1940.
6. Kodicek, E.: Estimation of Nicotinic Acid in Animal Tissues, Blood and Certain Food-stuffs. 2. Applications, *Biochem. J.* 34: 724, 1940.

A SIMPLE METHOD FOR THE DETERMINATION OF SERUM PROTEIN*

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THE Kjeldahl digestion method or one of its modifications is still the standard method for the determination of protein concentration in blood serum. The accuracy is adequate for most purposes, but the test is time-consuming. The clinical significance of the concentration of blood proteins has led to numerous investigations studying the significance of variations in blood protein level and methods of correcting hypoproteinemia (Bailey,² Allen,¹ Moon,⁸ Shuman and Jeghers.¹² The search for simple, yet accurate, methods for determining blood protein concentration has, therefore, assumed importance.

The nephelometric method of Reiss¹¹ represents an early attempt at simplification. The technique is quick and easy to learn, but its accuracy, particularly in nephrosis and lipemia, has been seriously questioned by Linder, Lundsgaard, and Van Slyke,⁷ and Guilanmin, Wahl, and Laurencin.⁶ Fishberg and Dolin⁵ suggested that the buffer action of blood is normally directly proportional to its protein concentration. They proposed a method of determining blood protein concentration, therefore, by measuring the resulting hydrogen-ion concentration of the blood after allowing it to react with a standard amount of acid. The results are reported to check very closely (within 0.5 per cent) with the Kjeldahl method. Its limitations are obvious in pathologic states in the presence of acidosis, alkalosis, or base depletion.

Moore and Van Slyke⁹ demonstrated a close, practically linear, relationship between the specific gravity of blood and its protein concentration. They showed that the specific gravity of blood represented the protein level more closely than did its refractive index and was influenced less by variations in lipid concentration. The specific gravity in their experiments was determined most conveniently by means of a specially constructed pyknometer. Barbour and Hamilton³ had previously described a method for determining the specific gravity of blood by measuring its rate of fall through an immiscible fluid under standard conditions. Their technique was of sufficient accuracy to en-

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able detection of hemoconcentration in dogs after emotional excitement or injection of adrenaline, and of hemodilution after sectioning the splanchnic nerves. This method, with modifications, is being used extensively in clinical laboratories to determine the specific gravity of blood or serum and to compute therefrom the concentration of protein. The calculation depends upon the empiric formula $[P = 343 (G - 1.0070)]$ of Moore and Van Slyke,² where P = concentration of serum proteins in grams per cent, and G = observed specific gravity.

Sufficient evidence has accumulated to show that the specific gravity is an accurate index of the protein concentration of blood and serum provided it can be determined with accuracy to the fourth decimal place. Both the pycnometer method of Moore and Van Slyke² and the falling-drop method of Barbour and Hamilton,³ or its modifications, have been shown to be adequate. The former, however, requires experience with the pycnometer and the analytical balance. A volume of less than 2 c.c. of fluid to be tested impairs the accuracy of the method.

The falling-drop method is accurate and can be carried out with a single drop of blood or serum, but there are many possible sources of error and experience with relatively complex apparatus is required before determinations are dependable.

A modification of the specific gravity method for the determination of serum proteins has been devised and tested. It involves the use of calibrated hollow glass beads, each of a carefully predetermined density. This report embodies a description of the method and the results of experiments with it.

METHOD

Small beads (1.5 to 2 mm. in diameter) were blown from hard glass capillary tubing and flame-polished. They were made at random in large numbers and their densities were determined individually by matching them with carefully prepared solutions of potassium sulfate (K_2SO_4) of known specific gravity at room temperature. The bead which stayed wherever placed in the standard solution without rising or falling in thirty seconds was catalogued as representing that particular specific gravity. The values were checked at least twice. Twenty-one such beads were selected as representing specific gravities of 1.0350 to 1.0172 (or serum protein concentrations of 9.60 to 3.50). Beads can thus be made to represent any desired range of protein values.

To determine the specific gravity of an unknown fluid, a bead thought to approximate the unknown specific gravity is selected and with bone-tipped forceps is placed below the surface of the unknown fluid. If the bead rises, it is replaced by one of greater density, and vice versa if it falls. If the bead shows no motion during an observation period of thirty seconds, it is taken to represent the specific gravity of the fluid tested and its protein content, in the case of blood or serum, is calculated by means of the Moore and Van Slyke formula. The test can be done in small glass test tubes of 5 mm. diameter and any convenient height. It is possible to test 0.5 c.c. of blood or even less.

RESULTS

I. The accuracy of the method in determining the specific gravity of liquids was tested by comparing its results with those obtained by the use of the pycnometer described by Moore and Van Slyke. Table I represents a comparison of specific gravities obtained by the two methods for solutions prepared by adding varying amounts of powdered glucose to aqueous solutions or to blood serum. The close agreement of the two methods is evident, the maximum difference being 0.0009; the average, 0.00047, represents for serum a protein concentration of 0.16 gram per cent. It may be concluded, then, that the bead method for determining the specific gravity of serum is sufficiently accurate for clinical purposes.

TABLE I

SPECIFIC GRAVITY OF MODIFIED* SERUM AS DETERMINED BY THE GLASS BEAD AND THE MOORE AND VAN SLYKE PYCNOMETER† METHODS (Including Figures in Table IV)

NO.	BEAD	PYCNOMETER	DIFFERENCE BEAD-PYCNOMETER
1	1.0274	1.0278	-0.0004
2	1.0278	1.0280	-0.0002
3	1.0283	1.0292	-0.0009
4	1.0312	1.0308	+0.0004
5	1.0265	1.0261	+0.0004
6	1.0275	1.0270	+0.0005
7	1.0279	1.0276	+0.0003
8	1.0291	1.0283	+0.0008
9	1.0300	1.0293	+0.0007
10	1.0253	1.0254	-0.0001
11	1.0210	1.0212	-0.0002
12	1.0225	1.0226	-0.0001
13	1.0241	1.0244	-0.0003
14	1.0269	1.0276	-0.0007
15	1.0281	1.0289	-0.0008

Average difference in specific gravity = $0.00047 = 0.16$ per cent protein. (Specific gravity 0.0001 = 0.0343 per cent protein.)

*Serum modified by adding solid glucose or distilled water.

†The pycnometer used here had a capacity of 10 c.c.

II. The serum protein concentration was determined in eighty-eight specimens of blood by the micro-Kjeldahl method and by the bead method described above. Fifteen more specimens were determined in duplicate. The thirty specimens were submitted for determination by both methods as unknowns.

Table II compares the results obtained by the micro-Kjeldahl and the bead specific gravity method in twenty representative determinations. The close agreement of the two methods is striking. The maximum difference between the results obtained by the bead and micro-Kjeldahl methods is 0.8 Gm. per cent. This difference appeared in only two of the 118 determinations. In two others the difference was 0.7 Gm. per cent, in one it was 0.6 Gm. per cent, and in nine it was 0.5 Gm. per cent. The average difference was 0.25 Gm. per cent.

Table III shows the results obtained in ten representative specimens out of fifteen which were determined as unknown duplicates by the two methods. It will be seen that closer checks, as a rule, are obtained by the bead method than by the micro-Kjeldahl digestion method.

TABLE II

SERUM PROTEIN CONCENTRATIONS (GRAMS PER CENT) IN TWENTY REPRESENTATIVE SPECIMENS DETERMINED BY THE MICRO-KJELDAHL AND THE GLASS BEAD METHODS

NO.	MICRO-KJELDAHL	GLASS BEAD	NO.	MICRO-KJELDAHL	GLASS BEAD
1	6.1	5.7	11	2.7*	<3.5†
2	4.3	4.7	12	4.5*	3.7
3	6.4	6.4	13	6.6	6.0
4	5.5	5.4	14	4.9	5.2
5	7.6	7.5	15	7.6	7.8
6	5.0	4.8	16	6.8	6.0
7	9.6	8.9	17	4.3	3.8
8	4.1	4.3	18	8.0	7.9
9	7.0	7.0	19	3.7*	3.9
10	4.4*	4.5	20	3.6*	3.7

*From patients with nephrosis.

†This represented the lightest bead in the set.

TABLE III

SERUM PROTEIN CONCENTRATION (IN UNKNOWN DUPLICATES) DETERMINED BY THE MICRO-KJELDAHL AND THE GLASS BEAD METHODS

NO.	MICRO-KJELDAHL	GLASS BEAD
1	6.1	6.4
	6.4	6.4
2	6.3	6.0
	6.6	6.0
3	5.2	5.6
	5.7	5.6
4	5.2	5.4
	5.5	5.6
5	5.4	5.6
	5.6	5.6
6	6.4	6.4
	6.8	6.4
7	4.8	5.5
	5.2	5.7
8	6.8	6.5
	6.8	6.6
9	7.6	7.5
	7.6	7.5
10	5.5	5.7
	5.5	5.7

TABLE IV

EFFECT OF VARYING THE GLUCOSE CONCENTRATION ON THE SPECIFIC GRAVITY OF BLOOD SERUM

GLUCOSE CONCENTRATION (MG. %)	SPECIFIC GRAVITY		SERUM PROTEIN DIFFERENCE	
	BEAD	PYKNOMETER	BEAD	PYKNOMETER
100	1.0265	1.0261	0	0
200	1.0267		0.07	
330	1.0275	1.0270	0.34	0.31
500	1.0278		0.44	
570	1.0279	1.0276	0.48	0.52
790	1.0291	1.0283	0.89	0.76
1,050	1.0300	1.0293	1.20	1.10

III. The effect of glucose concentration on the specific gravity of blood serum. The glucose concentration of normal blood serum was varied by the addition of powdered glucose and the specific gravity of the solution was determined by the bead method in seven instances. In five of these, it was determined also by the pyknometer method. The results are shown in Table IV and graphically in Fig. 1, in which the specific gravity difference is converted to the serum protein

concentration difference in grams per cent. Reference to these data demonstrates a practically linear error in the estimated serum protein concentration introduced by variations in the glucose concentration of the blood serum. This observation is of significance clinically for blood protein determinations done in patients with diabetes mellitus.

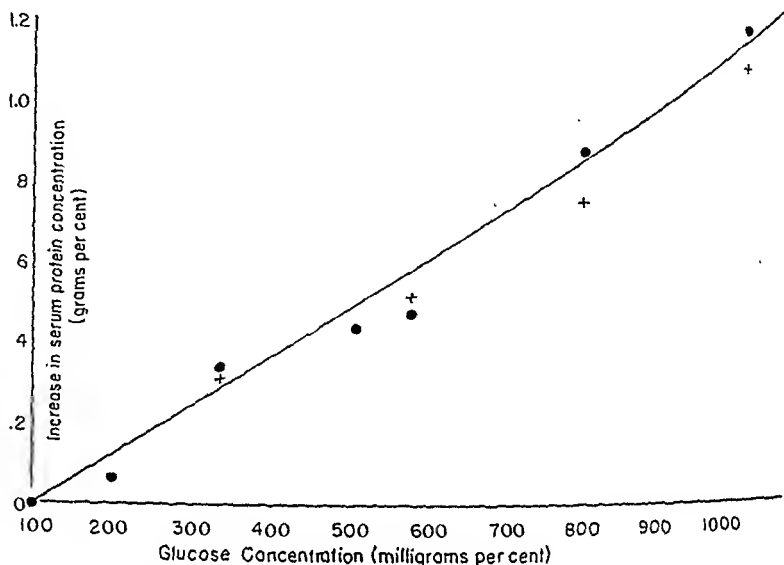


Fig. 1.—Graph relating glucose concentration of serum to specific gravity. Ordinates: specific gravity recorded as protein concentration; abscissae: glucose concentration; crosses: values obtained by pycnometer method; dots: values obtained by glass bead method.

DISCUSSION

The foregoing data demonstrate the practicability of a method for the determination of serum proteins, depending upon the virtually linear relationship between specific gravity and protein concentration. The specific gravity is accurately measured by means of hollow glass beads of previously determined density. Bing⁴ described a specific gravity method for determining serum protein by the use of a single bead representing a specific gravity of 1.0158 (protein concentration of 3.0 Gm. per 100 c.c.). Three cubic centimeters of serum are used and diluted with 0.9 per cent sodium chloride until the bead neither rises nor falls. At room temperature (20° C.) the diluted volume represents the protein concentration of the serum. The accuracy of the method depends in part upon the accurate delivery of 3.0 c.c. of serum. An error of 0.1 c.c. will introduce an error of 3.4 per cent in the total protein concentration. If smaller volumes of serum are used, the error will be correspondingly greater. It is convenient that it involves the use of only one bead. This advantage over the method described here loses significance when it is considered that once a set of beads is prepared, it can be used indefinitely and the necessity of volumetric instruments and diluting fluid is obviated.

The accuracy of any method depending upon the relationship between specific gravity and serum protein concentration could be influenced by a number of factors. The effect of changes in the albumin-globulin ratio was studied

by Nugent and Towle.¹⁰ They observed no significant effect of varying the albumin-globulin ratio of beef serum. Increases in the lipid concentrations likewise have relatively little influence on the specific gravity of serum (Moore and Van Slyke). The slight shifts that take place in the concentration of blood solids when the protein level changes have been found to be of no practical significance (Weech, Reeves, and Goettseh¹³). The data in Table IV show that abnormal concentrations of blood glucose may introduce a significant error in the protein concentration computed from the specific gravity. Sufficiently accurate corrections may be made by deducting 0.1 Gm. per cent protein for each increment of 100 mg. per cent glucose.

Certain precautions should be observed in determining specific gravity by the method described here. The beads are best kept in a padded partitioned box and handled with bone-tipped forceps. The blood or serum is placed in small glass tubes held vertically in a plasticine base. Determinations are done at room temperature. To avoid surface tension effects, the bead is placed in the solution directly below the surface. An air bubble may be carried down with the bead and should be carefully shaken off when present. Currents are sometimes set up in the fluid by the mere introduction of the forceps into it, particularly when tubes of larger diameter are used. If the bead is observed for thirty seconds, however, the effect of currents will have been spent. The beads, of course, should be kept clean, and this is easily accomplished by rinsing them in distilled water immediately after removing them from the blood or serum.

The method for blood protein determination presented in this report is offered as a quick, simple, yet accurate, one. No contention is held that this method is more accurate than the other specific gravity methods or the Kjeldahl method, but, since the technique is simple and sources of error are minimized, it will find greater adaptability in clinical research. For ordinary clinical purposes it is hardly necessary to have more than four calibrated beads representing serum protein concentrations of 5.0, 6.0, 7.0, and 8.0, respectively. With experience, it becomes simple to interpolate between beads and read values to within 0.25 Gm. per cent. Greater accuracy in the clinic is seldom required.

SUMMARY

1. A method for the estimation of blood or serum protein concentration is described, depending upon the determination of the specific gravity of the fluid by means of calibrated hollow glass beads.
2. The method compares favorably in accuracy with other reported techniques. Its advantages are its sensitivity, its simplicity, and its requirement of very small volumes.
3. The effect of glucose concentration on the determinations has been tested and a graph is presented (Fig. 1) from which corrections may be made.

REFERENCES

1. Allen, A. W.: Nutritional Factors Influencing Wound Healing (Internat. Abstr. Surg.), Surg., Gynec. & Obst. 69: 111, 1939.
2. Bailey, F. W.: Nonpenetrating Intra-Abdominal Injury, Ann. Surg. 109: 931, 1939.
3. Barbour, H. G., and Hamilton, W. F.: Blood Specific Gravity: Its Significance and a New Method for Its Determination, Am. J. Physiol. 69: 654, 1924.

4. Bing, J.: Serum Protein Determination by the Glass-Bead Method, *Acta med. Scandinav.* 96: 403, 1938.
5. Fishberg, E. H., and Dolin, B. T.: Determination of Serum Proteins, *Proc. Soc. Exper. Biol. & Med.* 28: 205, 1930.
6. Guillaumin, G. O., Wahl, R., and Laurencin, K. L.: Sur le dosage des albuminesériques. Comparaison de quelques résultats obtenus par pesée, par azotométrie, et par refractométrie, *Bull. Soc. Chim. Biol.* 11: 387, 1929.
7. Linder, G. C., Lundsgaard, C., and Van Slyke, D. D.: Concentration of Plasma Proteins in Nephritis, *J. Exper. Med.* 39: 887, 1924.
8. Moon, V. H.: Early Recognition of Shock and Its Differentiation From Hemorrhage, *Ann. Surg.* 110: 260, 1939.
9. Moore, N. S., and Van Slyke, D. D.: The Relationship Between Plasma Specific Gravity, Plasma Protein Content and Edema in Nephritis, *J. Clin. Investigation* 8: 337, 1930.
10. Nugent, R. L., and Towle, L. W.: The Specific Gravity of Synthetic Solutions of Serum Albumin and Serum Globulin, *J. Biol. Chem.* 104: 395, 1934.
11. Reiss, E.: Eine neue Methode der quantitativen Eiweiss Bestimmung, *Arch. f. exper. Path. u. Pharmacol.* 51: 18, 1904.
12. Shuman, H. H., and Jeghers, H.: The Value of Routine Blood-Protein Determinations, *New England J. Med.* 222: 335, 1940.
13. Weech, A. A., Reeves, F. B., and Goettsch, E.: The Relationship Between Specific Gravity and Protein Content in Plasma, Serum, and Transudate From Dogs, *J. Biol. Chem.* 113: 167, 1936.

NOTE ON A POSSIBLE SOURCE OF ERROR IN THE DETERMINATION OF SULFAPYRIDINE AND SULFANILAMIDE*

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THE twofold purpose of this paper is (1) to point out a possible source of error in the determination of sulfanilamide and sulfapyridine, and (2) to indicate that concentrations of procaine hydrochloride as low as 0.01 mg. per cubic centimeter of blood may be determined with a high degree of accuracy, thus making available a method for the study of the metabolism of procaine hydrochloride.

In the course of a series of sulfapyridine determinations on a patient to whom sulfapyridine was administered parenterally at a constant rate, it was observed that there was a rather marked fluctuation in the blood level of sulfapyridine. The fluctuation in blood level did not correspond to alterations in the dose or rate of administration of sulfapyridine. It was apparent, therefore, that some contaminant might possibly be responsible for the observed fluctuations. Upon inquiry it was found that it has been common practice for hospital interns to collect venous blood in syringes which had been used to anesthetize the skin, with 2 per cent novocaine (procaine hydrochloride), prior to venipuncture.

It should be noted that the color development of sulfapyridine and sulfanilamide depends upon diazotization of primary aryl amines. Procaine hydrochloride is a primary aryl amine and diazotization of its amine group, when the sulfapyridine reagents are added, according to the method of Marshall,¹ results in a pink color which is identical in tinctorial power with that obtained

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from sulfapyridine. As an indication of the magnitude of this source of error, it was found that after one drop of 2 per cent procaine hydrochloride is added to 5 c.c. of blood, a sulfapyridine value of 12.3 mg. per cent was found. There is no significant diminution in the value if the blood is allowed to stand at room temperature for two days.

An attempt was made to recover added procaine hydrochloride from blood to determine the lower limits of sensitivity of the method. Accordingly, procaine hydrochloride was added to blood in amounts sufficient to yield final concentrations of 1.0 and 2.0 mg. per cent. Precipitation of the blood was then carried out in 1:10 and 1:20 dilutions. The recovery of procaine hydrochloride from the blood containing 1.0 mg. per 100 c.c. was 1.02 mg. per cent in the 1:10 dilution of the blood, and 1.04 mg. per 100 c.c. in the 1:20 dilution of the blood, an error of plus 2 and plus 4 per cent. Recovery of procaine hydrochloride from blood containing 2.0 mg. per cent with 1:20 dilution was 2.14 mg. per cent, an error of plus 7 per cent. In these determinations the blood estimation was matched with a known procaine hydrochloride standard.

TABLE I

STANDARD SODIUM SULFAPYRIDINE MOL. WT. 271 (MG. %)	STANDARD SULFANILAMIDE MOL. WT. 172 (MG. %)	STANDARD PROCAINE HCl MOL. WT. 272 (MG. %)	DETERMINED PROCAINE HCl (MG. %)	CALCULATED PROCAINE HCl CONCENTRATION (MG. %)
-	0.20	0.20	0.130	$0.13 \times \frac{272}{172} = 0.20$
-	0.40	0.40	0.256	$0.256 \times \frac{272}{172} = 0.40$
0.20	-	0.20	0.200	$0.200 \times \frac{272}{271} = 0.20$
0.40	-	0.40	0.396	$0.396 \times \frac{272}{271} = 0.39$

It now seemed desirable to determine the tinctorial power of procaine hydrochloride as compared to sodium sulfapyridine and sulfanilamide. Standard 0.2 per cent and 0.4 per cent solutions of these substances were prepared. As may be seen in Table I, the color developed by procaine hydrochloride is practically identical with that developed by sulfapyridine—the molecular weights are nearly identical. When procaine hydrochloride is matched with standard sulfanilamide, procaine hydrochloride only develops about 64 per cent as much color. However, the ratio of the molecular weights of procaine hydrochloride and sulfanilamide is $\frac{172}{272} = 1.58$, indicating a 100 per cent recovery on an equimolecular basis. It is apparent then that on an equimolecular basis the tinctorial power of procaine hydrochloride is equal to that of sulfanilamide and sulfapyridine.

REFERENCE

1. Bratton, A. C., and Marshall, E. K., Jr.: A New Coupling Component for Sulfanilamide Determination, *J. Biol. Chem.* 128: 537, 1939.

OBSERVATIONS WITH A PHOTOELECTRIC HEMOGLOBINOMETER*

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PHOTOELECTRIC colorimeters have been adapted to many measurements including the quantitative estimation of hemoglobin. Reported observations indicate the feasibility of rapid and fairly precise hemoglobin measurement by the use of such instruments.¹⁻⁶ The present study was undertaken to survey the characteristics of a photometer designed particularly for use as a hemoglobinometer.

THE INSTRUMENT

The instrument used was a simple form of photoelectric colorimeter,[†] consisting essentially of a single photoelectric cell, a filter, a holder for solution containers, and a light source. The photoelectric cell was of the blocking layer type, the output being measured with a sensitive galvanometer built into the instrument. The lamp was energized by a pair of dry cells incorporated in the instrument case. The solution containers were commercially obtainable test tubes selected for uniformity in size of bore. The holder for the solution containers was of special construction to compensate for optical defects inherent in a cylindrical form of solution cell. Fig. 1 shows the general appearance of the instrument.

The apparatus was operated as follows: With the lamp off, the galvanometer needle was adjusted to a prescribed zero point at the right extremity of the scale by manipulating a small knob mounted on top of the instrument (Fig. 1). The lamp was then lighted, and a solution cell containing freshly distilled water was placed in the holder. This procedure caused movement of the galvanometer needle to the left. The needle was then adjusted to the left extremity of the scale by altering the lamp intensity by means of a rheostat provided on the front of the instrument (Fig. 1). The tube containing distilled water was then replaced by one containing an unknown solution of acid hematin prepared as noted below. The decreased transmission of light caused a deflection of the galvanometer needle to the right for a distance dependent upon the concentration of acid hematin present in the solution. For most consistent results the instrument was allowed to stand with the lamp lighted for about five minutes prior to the first reading. Before use tubes for solutions were carefully cleansed inside and out.

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†This photometer was an experimental model supplied by the Fisher Scientific Company, Pittsburgh, Pa.

EXPERIMENTAL PROCEDURE AND RESULTS

Five samples of beef blood, one of sheep blood, and two of human blood were examined. Upon each of these the following observations were made:

- a. Oxygen capacity by the manometric method of Van Slyke and Neill,⁷ carried out in duplicate or triplicate.
- b. Oxygen capacity by the blood iron method of Wong.⁸
- c. Photometer readings, after appropriate treatment, of whole blood and of dilutions with 0.9 per cent sodium chloride solution containing 80, 60, 40, and 20 per cent whole blood.

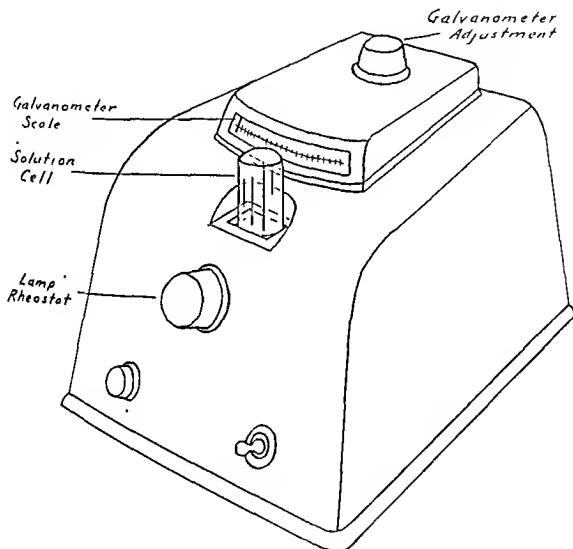


Fig. 1.—General view of photoelectric hemoglobinometer.

Samples of whole or diluted blood were prepared for photometric studies by diluting in a special pipette 20 c.mm. of the sample with 5 c.e. of tenth-normal hydrochloric acid. The resulting acid hematin solution was discharged into a solution cell and allowed to stand for five minutes. Although the color was not fully developed in this length of time, results were sufficiently consistent for practical purposes. Three or more photometer readings were made for each test, the galvanometer zero being reset, if necessary, between readings.

Table 1 shows the relationship of hemoglobin content, calculated from oxygen capacity, to photometer readings expressed in arbitrary units of the galvanometer scale. This relationship is shown graphically in Fig. 2, which is based on hemoglobin values obtained by the gasometric method. In the preparation of Fig. 2 hemoglobin contents of blood dilutions were calculated from the values determined for respective samples of whole blood.

TABLE I

RELATIONSHIP OF THE HEMOGLOBIN CONTENT OF BLOOD TO PHOTOMETER READINGS

BLOOD NO.	NATURE OF BLOOD	O ₂ CAPACITY		HEMOGLOBIN		PHOTOMETER READING UNITS
		GASOMETRIC (VOL. %)	BLOOD IRON (VOL. %)	GASOMETRIC (GM. %)	BLOOD IRON (GM. %)	
2	Sheep	15.45	16.40	11.6	12.3	36.0
3	Beef	16.49	16.87	12.4	12.7	35.3
4	Beef	21.31	22.10	16.0	16.6	40.2
5	Human	11.48	13.20*	8.6	9.9*	30.0
6	Human	8.97	10.12*	6.7	7.6*	25.8
7	Beef	19.99	20.50	15.0	15.4	38.6
8	Beef	22.66	23.25	17.0	17.5	42.2
9	Beef	22.29	22.47	16.7	16.9	41.3

*Patients supplying these samples were receiving iron medication daily.

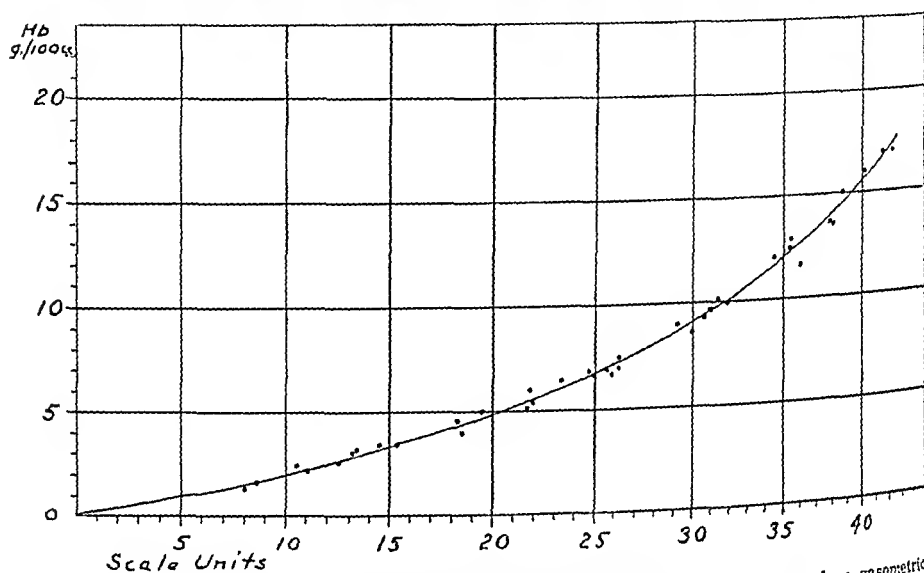


Fig. 2.—Relationship of photometer readings to hemoglobin concentration, based on gasometric hemoglobin determination.

DISCUSSION

The characteristics of this particular type of photometer favored its application to hemoglobin estimations. Operation was simple, requiring less than ten minutes for a complete determination. The amount of blood required was small and the final dilution was high, conditions which tend to minimize the effects of technical errors in sampling and dilution. As shown in Fig. 2, the calibration curve was quite flat, especially in the clinically important low hemoglobin range. Single readings were reliable within one unit on the galvanometer scale in approximately 90 per cent of consecutive trials. A deviation of one scale unit was equivalent to 0.3 Gm. of hemoglobin per 100 c.c. of blood below 5 Gm. total, 0.3 to 0.5 Gm. between 5 and 10 Gm. total, and 0.5 to 0.7 Gm. between 10 and 15 Gm. total hemoglobin content. Repetition of photometer readings was but a matter of seconds and naturally reduced the error of single readings.

A point of incidental interest was the comparison of hemoglobin measurements by gasometric and blood iron methods, as shown in Table I. The iron

method gave results consistently higher than those obtained by gasometric determination, the average difference amounting to 0.61 Gm. of hemoglobin per 100 c.c. of blood. The widest discrepancies occurred in the two human samples, both from anemic patients receiving large daily doses of iron. If these two samples are neglected, the average difference between results by the two methods was 0.45 Gm. per 100 c.c. We are inclined to agree with Haden⁹ that either method is suitable for normal bloods, and that the relative simplicity of the iron method is a definite advantage. However, it seems clear that the true respiratory value of the blood is more accurately portrayed by the gasometric measurement.

SUMMARY

1. The characteristics of a simplified form of photoelectric hemoglobinometer were studied by comparison of photometer readings with hemoglobin measurements according to the gasometric method of Van Slyke and Neill and the blood iron method of Wong.
2. The photometer employed was found to be suitable for hemoglobin measurements.
3. Single readings were reliable within 0.3 to 0.7 Gm. of hemoglobin per 100 c.c., depending on the total hemoglobin content of the sample. Multiple readings, occupying an insignificant amount of extra time, narrowed the limits of error.
4. Comparison of hemoglobin estimations by gasometric and blood iron methods was included in the study.

It is a pleasure to acknowledge the helpful suggestions of Dr. C. C. Guthrie.

REFERENCES

1. Dick, G. F., and Stevens, D. S.: A Photoelectric Hemoglobinometer, *J. A. M. A.* 114: 1065, 1940.
2. Vilter, S. P., and Johnston, R.: Photometric Determinations of Urea, Uric Acid, Creatinine, and Hemoglobin in the Blood of Scorbatic Guinea Pigs, *J. Nutrition* 13: 329, 1937.
3. Evelyn, K. A.: A Stabilized Photoelectric Colorimeter With Light Filters, *J. Biol. Chem.* 115: 63, 1936.
4. Armstrong, E. L., and Kuder, M. L.: The Limitations of Colorimetric Analysis by Present Methods, *J. LAB. & CLIN. MED.* 21: 181, 1935.
5. Holiday, E. R., Kerridge, P. M. T., and Smith, F. C.: Amount of Hemoglobin in Blood, *Lancet* 2: 661, 1935.
6. Kramer, K.: Determination of Oxygen Content and Hemoglobin Concentration of Hemoglobin Solutions and of Hemolyzed Blood by Photoelectric Method, *Ztschr. f. Biol.* 95: 126, 1934.
7. Van Slyke, D. D., and Neill, J. M.: The Determination of Gases in Blood and Other Solutions by Vacuum Extraction and Manometric Measurement. I, *J. Biol. Chem.* 61: 523, 1924.
8. Wong, S. Y.: Colorimetric Determination of Iron and Hemoglobin in Blood. II, *J. Biol. Chem.* 77: 409, 1928.
9. Haden, R. L.: Determination of Hemoglobin by the Iron Content Method, *J. LAB. & CLIN. MED.* 19: 406, 1934.

A TECHNIQUE FOR THE DETERMINATION OF PROTHROMBIN TIME*

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WITH the introduction of the effective use of vitamin K in the treatment of hemorrhage in jaundice by Warner, Brinkhous, Smith¹; Butt, Snell, Osterberg²; Dam and Glavind,³ the need for a practical and accurate method of determining the prothrombin content of the blood, now known to be related to vitamin K absorption, has assumed increasing importance.

The techniques for prothrombin determination have been limited to two accepted methods: that of Dam and Glavind⁴; which is extremely time-consuming and laborious; and that of Quick, which is more practical for clinical purposes, and the more widely used of the two. Even the method of Quick,⁵ however, is burdensome, since it does not include a stable thromboplastin, and therefore requires frequent renewal of the preparation.

During the past year a technique has been developed and successfully employed in our laboratories which eliminates the above-mentioned disadvantages of time and complexity, and so facilitates the increased use of prothrombin determination, that we feel it merits a detailed description.

The apparatus employed consists of a constant temperature water bath, set at 40° C., and an anatomic museum jar, measuring 25 by 21 by 9 cm., with sides of fairly uniform thickness, as the water tank. The heat source is a knife-blade heater connected to a mercury thermoregulator by means of a magnetic relay. The water between the heater and thermoregulator is kept in motion by a stirrer attached to the shaft of a slow motor, as illustrated in Fig. 1.

The reagents required are a potent thromboplastin extract and a 1/40 M calcium chloride solution. The thromboplastin is obtained by extraction from the brains of stillborn fetuses. To obtain consistent results with extracts prepared by this method, from a number of brains, the brain should not be kept in the refrigerator for more than thirty-six hours after death. To prepare the brain for extraction, the pial membranes and vessels are removed as completely as possible, and the brain is cleaned by immersing for a moment in cold water. It is then weighed, cut into small pieces, and put into a large mortar. An equal weight of pure sand is added and the mixture is ground with a pestle until it has an even creamlike consistency. To this mixture is added an approximately equal volume of normal saline and thoroughly mixed. The material is then centrifuged until the supernatant fluid is only slightly cloudy. This takes approximately one to one and a half hours. The fluid is then poured off, filtered through cotton, sealed in 5 c.c. vials, and kept frozen by storage in a freezing chamber. Thromboplastin prepared from a single brain and stored in

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this manner has been kept for six months without any signs of deterioration in potency, and has been sufficient in quantity for approximately 200 determinations.

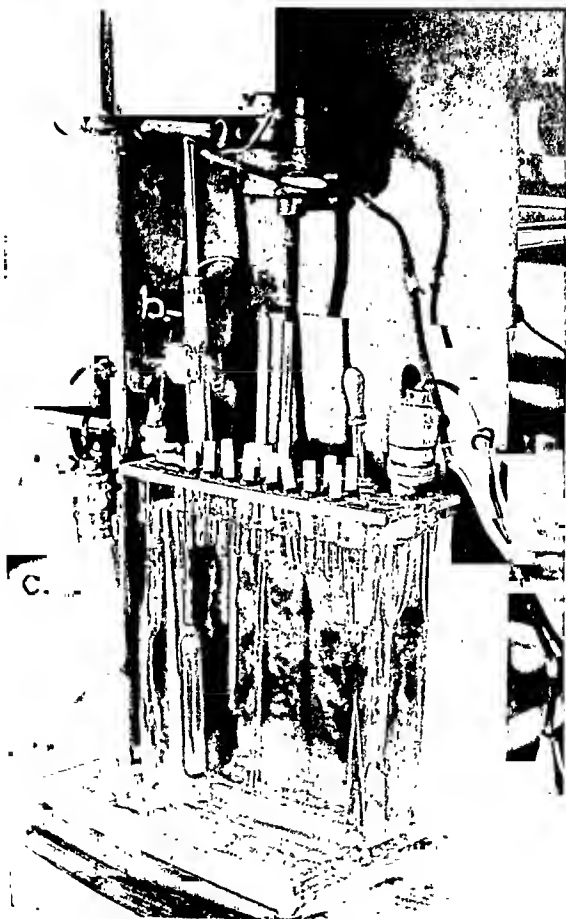


Fig. 1.—*a*, Knife blade heater; *b*, mercury thermoregulator; *c*, light source.

In preparation for the test a vial of the thromboplastin extract is defrosted in warm water, and an equal volume of $1/40$ M calcium chloride solution is added to it. This mixture is then immersed in the water bath, which is kept at

40° C., and allowed to incubate for thirty to forty-five minutes, after which time it will be noted that a flaky white material has been precipitated, and the supernatant fluid is almost clear. This fluid is centrifuged at high speed (about 2,000 r.p.m.) for ten minutes until clear, and the clear fluid is then returned to the water bath.

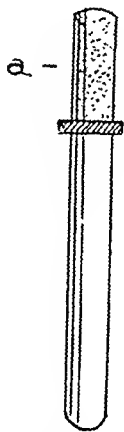


Fig. 2.—a, Ground glass surface for labeling.



Fig. 3.

To collect the blood sample, 15 c.c. calibrated centrifuge tubes, containing 0.5 c.c. of 3 per cent sodium citrate solution, are used, to which 5.5 c.c. of blood are added, giving a total of 6 c.c. The plasma and cells are separated by centrifuging at 2,000 r.p.m. for five minutes, as soon as received, the plasma being kept in the refrigerator until used for the determination. Blood collected and treated in this fashion will yield a plasma which will give reliable results even after twenty-four hours, in contrast to the thirty minutes previously considered safe.

To determine the prothrombin time, 0.1 c.c. of plasma is pipetted into special round bottom tubes (Fig. 2), which in the meantime have been allowed to come to the temperature of the water bath. The plasma is incubated in the water bath for five minutes. Approximately 0.2 c.c. of the calcium chloride thromboplastin mixture is drawn up into the aspirator (Fig. 3) and is quickly expelled into the tube containing the plasma. At the same time the stop watch is started. The tube is viewed at right angles to the source of illumination, and when clotting appears, manifested by the beginning of opacity, the watch is stopped. (The reading of opacity is greatly facilitated by painting the back of the water bath black.) The time noted is the prothrombin time. By this method normal human plasma has a range of 10 to 13.3 seconds and an average of 12.1, with a mean variation of 0.7 second. With but little experience one can repeat readings on a given sample of plasma within 0.6 second. For routine work three readings are done; these should agree with each other within the limits of one second.

Recently I have used a thromboplastin extract prepared from brains as those described, but have varied the method somewhat to eliminate the necessity for adding calcium chloride at the time of determination. In this case, the extraction medium is a solution of 1/40 M calcium chloride in normal saline. A mixture of these two substances is centrifuged and stored in vials as described above. If, when doing the determination with this extract, one desires a greater volume of thromboplastin extract, addition of an equal volume of normal saline will not diminish its potency. With this preparation it is important that the extract incubate in the water bath for forty-five minutes before centrifuging. Results with this extract on normal plasma are somewhat higher than those described. By this method normal human plasma has a range of 15.3 to 24.9 seconds and an average of 20.8, with a mean deviation of 1.9 seconds. This extract has proved as stable and reliable as the one originally used.

Experiments with a thromboplastin extract in which an equal volume of 1/40 M calcium chloride solution was added to the thromboplastin after its extraction with normal saline and then stored as mentioned above, yielded an extract similar in potency to the one extracted with 1/40 M calcium chloride in normal saline. This preparation, however, showed evidence of deterioration after two months. The use of stillborn fetus brains has yielded a thromboplastin superior in activity and stability to that extracted from the brains of embryo and adult cattle and rabbits, as well as adult human beings.

SUMMARY

1. The preparation of a stable and potent thromboplastin extract which eliminates the repeated extraction of thromboplastic material is described.
2. A description of a simple, inexpensive, and reliable technique and apparatus for the determination of prothrombin time is presented.

Sincere thanks are extended to Mr. Bernard Sobel, of the Division of Chemistry, for his aid in assembling the apparatus.

REFERENCES

1. Warner, E. D., Brinkhous, K. M., and Smith, H. P.: Bleeding Tendency of Obstructive Jaundice: Prothrombin Deficiency and Dietary Factors, *Proc. Soc. Exper. Biol. & Med.* 37: 628, 1938.
2. Butt, H. R., Snell, A. M., and Osterberg, A. E.: The Use of Vitamin K in Treatment of the Hemorrhagic Diathesis in Cases of Jaundice, *Proc. Staff Meet., Mayo Clin.* 13: 74, 1938.
3. Dam, Henrik, and Glavind, Johannes: Vitamin K in Human Pathology, *Ugesk. f. læger.* 100: 248, 1938.
4. Dam, Henrik, and Glavind, Johannes: The Clotting Power of Human and Mammalian Blood in Relation to Vitamin K, *Acta med. Scandinav.* 96: 108, 1938.
5. Quick, A. J.: The Nature of Bleeding in Jaundice, *J. A. M. A.* 110: 1658, 1938.

THE IMPORTANCE OF TEMPERATURE FOR COLORIMETRIC DETERMINATIONS*

WITH SPECIAL REFERENCE TO NESSLER'S REACTION

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COLOR reactions for determining biological constituents are widely used in this country. In most cases the colored solutions to be compared display a more or less colloidal behavior.

A few incidental observations showed that for one of these colorimetric methods, the yellow color produced by adding Nessler's reagent to ammonium sulfate solutions obtained by digesting urine with sulfuric acid, is very dependent upon the temperature.

As shown by many investigators the color produced by Nessler's reagent in solutions of ammonia goes parallel to the amount of nitrogen whether the ammonia was obtained by distillation or the nitrogen solution digested by sulfuric acid was immediately used for the test (direct nesslerization).

To avoid complications resulting from the digesting of organic material in the way commonly used for nitrogen determinations, the following experiments were made with pure ammonium sulfate solutions.

Into each of two large test tubes graduated to 35 and 50 c.c., 5 c.c. of ammonium sulfate solution containing 1 mg. of nitrogen were pipetted, 1 c.c. of sulfuric acid solution (1 c.c. of concentrated sulfuric acid, 1 c.c. of water) added and made up to 35 c.c. with water. The tubes were kept in ice water for fifteen minutes. Nessler's reagent just taken from the refrigerator was slowly added to the mark 50, mixed, and the tubes left in the ice water for three more minutes. After this time one tube was left in the ice water, the other was taken out and kept at room temperature.

Both cups of the colorimeter were filled with the solution in ice water. Readings were in the beginning 30:30; after being left in the cups ten minutes, 30:29.7; after twenty minutes, 30:29.6; after thirty minutes, 30:29.7. Thus the readings were practically constant for the solutions, ice cold in the beginning being warmed together to room temperature by standing in the cups.

The solution (now at room temperature) was left in one of the cups and the solution in the other cup replaced by new solution kept at room temperature but the reading did not change, 30:29.5, 30:29.8. Thus with both solutions at the same temperature the reading is correct and stays that way.

When the room-warm solution was left in one cup and cold solution (from the test tube in ice water) was substituted in the other cup, the reading was

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30:17.5 (30 being the layer of the ice-cold solution). While the solutions were in the cup, the temperature of the cold solution slowly went up, the reading went down to 30:24, and after thirty minutes to 30:29, practically reaching the correct reading.

If the cups were emptied and one cup was filled with ice-cold solution and the other with solution at room temperature, the reading was 30:17.5 and went down to 30:25, and finally to 30:30, when the solutions had reached the same temperature. These experiments were duplicated in manifold ways. Parts of the same nesslerized solution showed the phenomenon, if one-half was kept cool, the other half at room temperature, and the difference in reading could almost be reversed by reversing the temperature of the solutions. The changes of the optical transmission of these solutions were hardly visible to the naked eye; the solutions looked clear and transparent. It may be added that there was no difference whether a protective colloid was added or not.

The explanation of these findings is, of course, given by the colloidal status of the solutions, the optical transmission being decreased by raising, and increased by lowering the temperature. As far as can be seen in these few experiments this process is, at least for a certain time, more or less reversible. Theoretical questions cannot be discussed in this paper.

Some other color reactions, such as the determination of phosphorus with hydroquinone-molybdic acid (Bell and Doisy) and the determination of glucose with the Benedict method did not show this influence of temperature.

Using Nessler's method, whether with or without preceding distillation and with or without protecting colloid, care must be taken that the temperature of the solutions in the cups is the same. For practical purposes the best procedure seems to be to add cold Nessler's reagent to the cold solutions to be compared, add if desired the protective colloid, mix, leave the solutions in the cold for ten minutes, take them out and let them stand for twenty minutes at room temperature. In this way the solutions have the same temperature and are aged to the same degree. For each reading not only the solution to be tested but also the standard solution may be put in the cups at the same time, thus avoiding the danger of unequal warming by the light source.

MEDICAL ILLUSTRATION

CLINICAL LABORATORY PHOTOGRAPHY WITH A NEW METHOD OF PREPARING PHOTOMICROGRAPHS*

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THE value of a simple and inexpensive method of routine photography for use in the modern clinical laboratory need not be emphasized. Indeed, there are few institutions possessing aggressive staffs which have not recognized the value of such facilities and attempted a solution. Few hospitals that are not directly associated with teaching institutions or research laboratories have the services of trained photographers for their requirements. The responsibility is usually relegated to the pathologist or radiologist who far too frequently has not had the opportunity for study or experience in this field. As a result, the prospect of starting such a department is alarming from the triple viewpoint of expense, time involved, and results produced.

For those laboratories and institutions already possessing cameras and equipment suitable for gross and photomicrographic work, the methods outlined here may be of little value. There is no denying the fact that better photographs are easier to obtain by using more expensive precision apparatus and larger film sizes or plates; however, it is possible to work with 35 mm. film and obtain excellent results. It might be found advantageous in terms of simplicity and economy to consider the procedure given here for routine photomicrographic work. Hellwig¹ has stressed the need for such procedures and given a method. To the best of my knowledge there has been no prior publication dealing with photomicrographs by the method here described, yet from its very simplicity it must have been used.

GROSS AND CLINICAL PHOTOGRAPHY

Equipment.—The equipment listed here is for the convenience of those desiring such information. There are many other similar cameras and apparatus available which undoubtedly will do equally as satisfactory work. The following equipment can be purchased for less than one hundred dollars.

1. Argus C₂ 35 mm. camera, or for those desiring photoflash equipment the C₃ is available at a slight additional cost.
2. The Arguscope, which essentially gives accurate ground-glass focusing for close work.
3. Photoelectric cell type light meter.

*From the Laird Memorial Hospital, Department of Pathology, Montgomery.
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4. Any 35 mm. film enlarger. A considerable saving can be effected by using an enlarger that will accommodate the camera lens.

Technique.—For photographing patients, equipment, and large specimens, or for use in the necropsy room, the camera alone is satisfactory. It is equipped with a coupled range finder, and accurate focusing from three feet can be done easily.

The Arguscope is invaluable in photographing surgical, necropsy, or other small objects. Full instructions are supplied by the manufacturers and are extremely simple. Photographs may be taken covering fields from $8\frac{1}{2}$ by $12\frac{3}{4}$ inches down to 1 by $1\frac{1}{2}$ inches, and at distances from $19\frac{1}{2}$ inches down to $3\frac{3}{4}$ inches from the object. The use of the ground-glass attachment for critical focusing and composition enables the routine production of prints of excellent quality.

Mallory² gives directions for preparing a tank devised by Bitterman for photographing specimens. The use of a tank is satisfactory, although in using 35 mm. film a smaller tank, that is, one that encompasses a field equal to the area covered by the camera, is also satisfactory. The purpose of photographing specimens under water is to eliminate annoying high lights. A defect that develops from lighting the specimen while under water is flatness or a lack of modeling. It is advisable, therefore, that one of the lights be placed at an acute angle to produce modeling. A far better method of eliminating high lights and still preserving the modeling is to light the specimen through a tracing cloth screen as described by Schmidt and Haulenbeck.³

The background of gross specimens may be made white on the print by using an auxiliary light beneath a glass base upon which the specimen may rest. The same thing can be accomplished by opaquing the background in the negative. However, the former method is easier and better. A more complete discussion of this subject will be found in another publication.⁴

The use of a celluloid ruler provides an easy method for critical focusing and in addition gives an easily interpreted scale for determining the size of the specimen from the prepared photographs. The ruler should be placed one-third distance from the top of the specimen, or two-thirds distance from the background. After focusing on the ruler, the diaphragm is closed as far as possible to obtain the maximum depth of focus. When the specimen is placed on a clear glass background with a light beneath, a transparent celluloid ruler is most effective.

Film, including the 35 mm. size, is available in many different types of emulsions. I have found the Eastman Panatomic X, processed in the Eastman DK-20 developer, to be the most satisfactory for general use in clinical and gross specimen photography. This 35 mm. film may be purchased in handy cartridges already prepared by the manufacturer, although a considerable saving may be effected by the use of bulk film. The almost routine photographing of surgical specimens and the attaching of prints to the completed reports necessitate development of the negatives fairly soon after their exposure. By purchasing film in bulk quantities and loading old cartridges with film lengths between twelve and fifteen inches, one is able to obtain eight to ten exposures for

each strip, instead of waiting several days as is often necessary to complete the exposures on a long roll of film. The DK-20 developer can be used for a considerable number of such strips without appreciable deterioration.

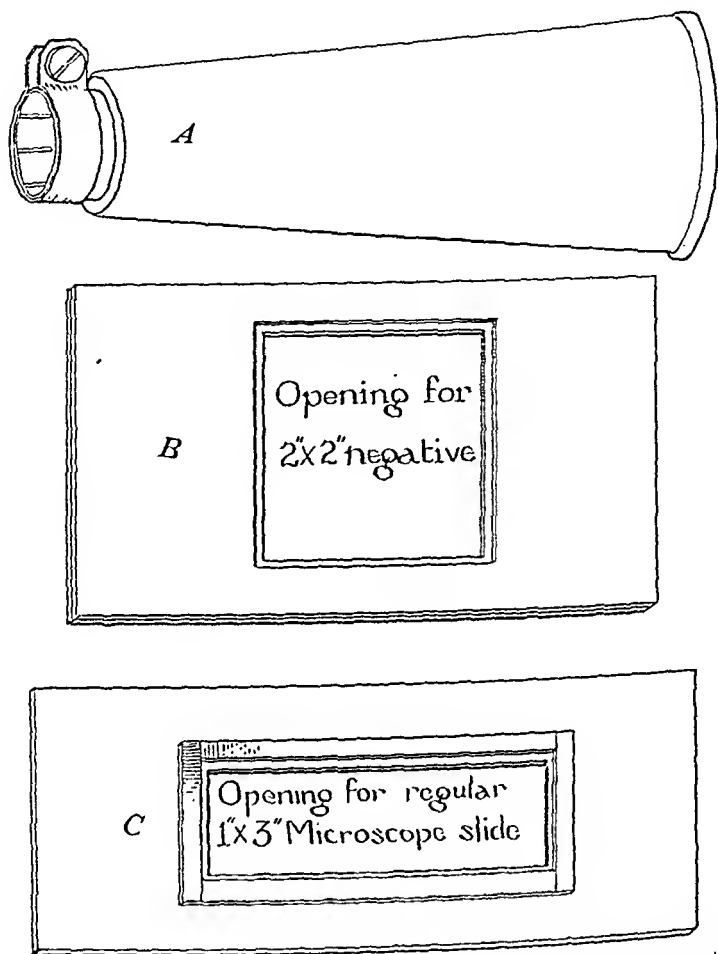


Fig. 1.—A, Cone-shaped adapter for fitting on microscope to hold the lantern slide. B, Cardboard enlarger carrier to hold 2 by 2 inch negative. C, Enlarger to hold 1 by 3 inch microscope slide.

Miniature camera work is extremely well adapted to the use of color film, and Kodachrome transparencies (type A, indoor) add greatly to the value of the work performed. For viewing these transparencies a regular 2 by 2 inch projector is available, or the larger-sized projectors may be converted by a simple attachment to handle the 2 by 2 inch sizes. In the event these adapters are used on the large projectors, it is advisable also to change the lens of the large projector to one of a shorter, proper focal length. In this manner the image on the screen will be the same size as the image projected from the larger slide, provided the projector is at the same distance from the screen. In changing back to the larger slide it becomes necessary to change the lenses again.

An attractive method of displaying these 2 by 2 inch transparencies, and likewise keeping them, has been developed by the Filmdex Corporation of McKeesport, New York.

Extremely satisfactory colored photomicrographs for projection or use as transparencies for museum purposes can be prepared very simply from black-and-white lantern slides by the method described by Putnam.⁵ This method is also applicable to the preparation of transparencies by means of Eastman Translite enlarging paper.

PHOTOMICROGRAPHS

The Arguscope can be used in photomicrographic work if desired. The results are variable; however, the method given here has proved satisfactory.

The equipment required is minimal, provided one possesses a microscope and a lamp. Beyond these, a darkroom, the adapter to be described, and a supply of 2 by 2 inch medium lantern slides are all that are necessary. The adapter consists of a cone (Fig. 1A) which fits over the monocular tube and accommodates the ocular. The tube shown in the illustration measures $5\frac{1}{2}$ inches in total length and $1\frac{7}{8}$ inches in diameter at the top. However, these tubes can be made in different lengths according to the additional magnification or reduction that is desired in relation to the objective and ocular used. By doubling the length of the tube the magnification is increased about one-fourth. Of course, if a bellows is used in place of the tube, the image can be varied to fill out the film with a specific composition. The exact magnification can then be determined by means of a stage micrometer. The inside of the adapter or bellows is painted dull black to prevent light reflections. These adapters are easily prepared or can be obtained from H. F. Holmes, Montgomery, W. Va., for two dollars. A bellows and the necessary supports would be more expensive.

In use the adapter is fitted to the microscope, as shown in Fig. 2, and the top of the cone is covered by a piece of ground glass, measuring 2 by 2 inches, with the ground surface down. This is easily prepared by removing the emulsion from a 2 by 2 inch lantern slide and then rubbing briskly with a valve grinding compound, procurable at any garage, until an evenly ground surface is obtained. The placing of penciled cross lines in the center enables one to focus accurately on the ground surface.

The lamp is tilted or twisted until the beam of light from the condensing lens falls squarely on the center of the microscope mirror. The condensing lens in the best lamps for photomicrography is adjustable, so that the beam can form a small spot on the mirror. The smaller this spot the more intense is the light, thus making short exposures at high magnifications possible. Lamps that are more suitable for photomicrography are the ribbon filament, the arc, the Point-o-lite, and the new photomicrographic lamp of Bausch & Lomb Optical Co and General Electric Co. The arc is the most common in use, but the ribbon filament lamp is rapidly taking its place. The Point-o-lite is an excellent light source but somewhat difficult to use. The new photomicrographic light is not yet in common use and its value, therefore, cannot be judged. In any event, the beam of light is centered accurately, the condenser of the microscope is brought into focus, and the objective filled with light; these procedures are necessary in all photomicrographic work and are accomplished easily. A slide is inserted and the microscope is brought into focus on the ground glass; the focus is checked by means of a hand lens. The field desired to be reproduced

is selected; obviously, it should be free from defects or artifacts. The microscope lamp is turned off, the ground glass is removed, and a 2 by 2 inch film or plate is put in place.

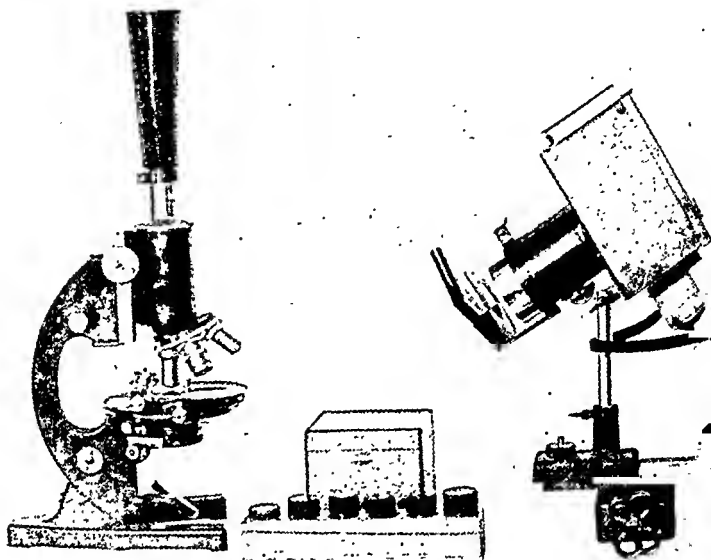


Fig. 2.—Microscope showing the adapter in place. In this picture the ribbon filament 6 volt lamp is used. This lamp has a condensing lens for spotting the light on the mirror of the microscope and a holder for two filters which are seen in place. Other light sources are discussed in the text. The lenses used in making the pictures seen in Fig. 3 are also shown. The box is for holding the various color filters.

In considering the sensitized negative materials most suitable for photomicrography, one should choose the type of film or plate on the basis of contrast desired and its sensitivity to the colors to be recorded. Most workers find it desirable to use a plate of high available contrast, high resolving power, and general sensitivity to all colors. The Wratten M plate for all types of photomicrography that do not require extreme sensitiveness is generally used. This plate, if properly employed, will produce negatives of a wide range of contrast, depending on the factors of varying the exposure and developing time. Photographic plates having only the original color sensitivity of the silver salts to ultraviolet, violet, and blue lights are technically considered to be "ordinary" plates (commercial and process), and may be developed in red light. When the sensitivity is extended by dyes into the green, the plates are orthochromatic. These, too, may be developed in a red light of low intensity. Panchromatic materials are sensitive throughout the whole visible spectrum and sometimes even beyond, into the near infrared.

With panchromatic material it is generally necessary to use a light filter between the light source and the condensing system of the microscope. It is best to select the desirable filter by visually inspecting the subject through filters until one is found that shows up the subject to best advantage. No one filter will fit all cases, nor can the filter be disregarded in every instance in

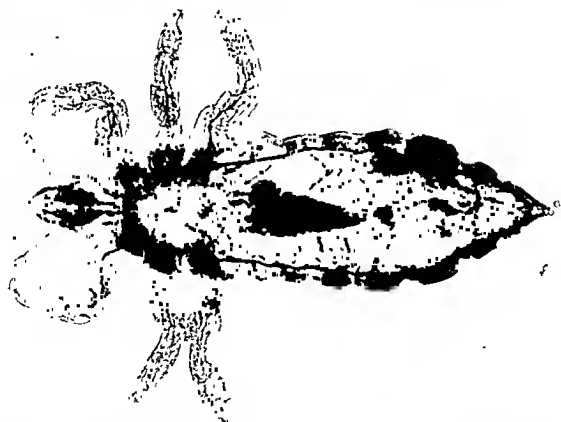


FIG. 3.—Photomicrographs showing s kidney tissue. A $\times 7$ ocular was used on objectives were as follows: A, Micro Tess Apochromat; D, $\times 20$ Apochromat; E, $\times 40$ lower photomicrograph of a parasite.

of the same area of magnifications of the Apochromat; C, $\times 10$ omat; G, A typical low

spite of the fact that a process or process panchromatic plate will at times give the desired contrast and detail in the resulting negative.

The book *Photomicrography*⁶ gives the following table which is of assistance in choosing a filter suitable for specific cases:

Use for blue stained preparations	a red filter
Use for green stained preparations	a red filter
Use for red stained preparations	a green filter
Use for yellow stained preparations	a blue filter
Use for brown stained preparations	a blue filter
Use for purple stained preparations	a green filter
Use for violet stained preparations	a yellow filter

In some cases it is necessary to use two filters and panchromatic material to obtain the best results. For example, a green and a yellow filter in combination often show up the hematoxylin-eosin stained section most satisfactorily.

All the foregoing photographic work can be done in a darkroom, provided the plate or film is not incased in a holder or magazine. Exposure of the film can be made by snapping the microscope light on and off; only a few trials are needed before one can accurately judge the correct exposure. Development is carried out in total darkness with the time and temperature method if panchromatic material is used; otherwise the ruby light is employed and some control over density is possible. It is also permissible to use lantern slide plates for making the negatives. These plates are very contrasty and correspond to a process emulsion. Furthermore, they are considered to be "color blind" in the sense they do not give true color values in black and white. This finished plate is a negative and may be used by contact printing to produce a print or, better yet, an enlargement. These enlargements are easily made by cutting a cardboard or plywood carrier to fit in place of the usual 35 mm. film carrier (Fig. 1B). There is a minimum of grain present in the lantern slide emulsion and enlargements of a number of diameters are possible (Fig. 3). Lantern slide positives may be prepared by contact printing on 2 by 2 inch slides, or by enlargement on the standard 3¼ by 4 inch lantern slides.

In taking photomicrographs by the method discussed, the size of the field encompassed is limited. With this in mind experiments were made to find a practical solution for getting around this difficulty. It was solved in the following manner:

A 35 mm. enlarger was used with a cardboard carrier cut so as to enclose in the insertion a standard glass slide, 1 by 3 inches, on which was mounted the tissue section. One can insert the carrier with the slide in the enlarger and project the entire image of the section on a 2 by 2 inch or a 3¼ by 4 inch lantern slide as a negative. No filters are necessary for an approximate record. However, if panchromatic negative materials and filters are used over the enlarger lens, better color renderings and contrasts will be obtained. Of course, such work must be done in total darkness, or while using a green safelight. The negative in either case should be sharp; by using it as outlined excellent prints may be obtained. This approximates very low power photomicrography in an extremely simple manner.

Photomicrographic negatives have been prepared on lantern slide plates from sections stained with hematoxylin-eosin, polychrome methylene blue, and

Masson's trichrome stain. Each of these methods of staining reproduces satisfactorily, and the use of panchromatic materials and filters has not been found necessary in all cases. In blood films, Wright and Strumia's stains have been used with excellent results.

GENERAL

An extremely interesting and relatively simple method for the coloring of photographs of gross specimens is given by Hopkins.⁷ A red to red-brown color on prints is obtained as follows: solution A, 45 grains of uranium nitrate and 10 ounces of water; solution B, 40 grains of potassium ferrieyanide and 10 ounces of water. Equal volumes of each solution are mixed, and 20 minims of glacial acetic acid are then added to each ounce of the mixture. Prints should be prepared in the usual manner, and thoroughly washed and dried. Artist's gum mastic is applied to all areas in which it is desired of obtaining red. The gum mastic dries rapidly. The print is washed in several changes of distilled water and toned in the solution with the depth of color visually controlled. The prints are then blotted and dried, after which the gum mastic may be removed with alcohol. A satisfactory blue may be added by covering all parts of the print except those which are desired to take the blue color. A solution consisting of the following ingredients is prepared: $\frac{1}{2}$ ounce of 10 per cent solution ferric ammonium, $\frac{1}{2}$ ounce of 10 per cent solution potassium ferrieyanide, and 5 ounces of 10 per cent solution acetic acid. The print is immersed until it assumes a dark greenish-blue color, after which it is washed thoroughly. The blue color is intensified by fixing in a hypo bath, and the gum mastic is then removed with alcohol. This method, though somewhat time-consuming and formidable at first glance, is surprisingly easy after a few trials, and the results are well worth the time expended. This subject is discussed more fully in another article.⁸

SUMMARY

Procedures suitable for routine photography in a clinical laboratory are discussed, and certain technical details are given. A method of preparing photomicrographs is outlined. Its value lies in the simple equipment necessary, the foolproof technique, and the quality of photomicrographs obtained. Attention is called to methods for preparing lantern slides of histologic specimens in color and for preparing prints of gross specimens in color that are suitable for use in one's own laboratory.

REFERENCES

1. Hellwig, H. H.: Routine Microfilming of Tumor Slides, *Am. J. Clin. Path., Tech. Supp.* 4: 95, 1940.
2. Mallory, F. B.: *Pathological Technique*, Philadelphia, 1938, W. B. Saunders Co., p. 385.
3. Schmidt, L., and Haulenbeek, J. B.: A Method of Photographing Gross Specimens, *J. Biol. Photographic A.* 1: 76, 1932.
4. Clarke, C. D.: Apparatus for Macrophotography, *J. Biol. Photographic A.* 2: 76, 1933.
5. Putnam, T. J.: A Simple Method of Preparing Lantern Slides of Histologic Specimens in Two Colors, *Arch. Path.* 28: 398, 1939.
6. *Photomicrography*, ed. 13, Rochester, 1935, Eastman Kodak Co.
7. Hopkins, A. A.: *The Scientific American Cyclopedia of Formulas*, ed. 29, New York City, 1925, Scientific American Publishing Co., p. 697.
8. *Color Photomicrography*, X-ray Bulletin and Clinical Photography, Rochester, 1927, Eastman Kodak Co. 3: No. 1, p. 3.

DEPARTMENT OF ABSTRACTS AND REVIEWS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

TYPHOID CARRIERS: Soluble Iodophthalein in Treatment of Carriers of Typhoid-Paratyphoid Group, Saphir, W., and Howell, K. M. J. A. M. A. 114: 1988, 1910.

A series of conservative measures, including the use of sulfanilamide, failed to render the stools of a carrier of *Bacterium paratyphosum* A free from these bacilli.

The oral administration of soluble iodophthalein was followed by immediate disappearance of the paratyphoid A bacilli from the stool. Repeated stool examinations extending over a seven months' observation period remained consistently negative for these organisms.

The results of a bacteriologic study in vitro as to the bactericidal action of soluble iodophthalein on the bacilli of the paratyphoid group did not entirely explain the curative action in a human carrier.

SULPHANILAMIDE, Antienzymic Nature of Bacteriostatic Action of, Mellon, R. R., Locke, A. P., and Shinn, L. E. Am. J. M. Sc. 199: 749, 1940.

The free amino group of sulfanilamide when incompletely oxidized becomes poisonous for catalase, as a result of which the toxic hydrogen peroxide produced by many pathogenic organisms is permitted to accumulate in their cultures.

In addition to catalase, other enzymes are adversely affected by the intermediate oxidation products of the sulfonamide compounds. As known so far, they are peroxidase, certain dehydrogenases, and nitrotase. The nutrition of the bacteria is thus seriously affected, and bacteriostasis results.

The effect of such bacteriostasis against pneumococci in vivo is to bring about their "dissociation" into culture phases, whose principal metabolic functions, including hydrogen peroxide formation and virulence, are so critically suppressed that the organisms fall easy prey to the destructive action of the phagocytes.

BRUCELLA, Studies on a Purified Antigen From, Morales-Otero, P., and Gonzalez, L. M. Am. J. M. Sc. 199: 810, 1940.

A method for the preparation of a purified protein derivative from brucella cells is described. The product obtained is of a fine consistency, light brown in color, and of a fairly constant chemical composition; it can be measured accurately and constitutes a good antigen.

The preparation has been used successfully as an antigen in complement fixation tests and in determining cutaneous hypersensitiveness to brucella in man and laboratory animals.

In man there is a direct relationship between contact with infected material or ingestion of infected raw products and skin reaction to purified brucella protein.

The incidence of positive reactors to the skin test in Puerto Rico, where *Br. abortus* infection is prevalent in cattle, was 24.7 per cent in contact groups, and 4.2 per cent in groups from the general population consuming raw milk from infected sources. On the island of St. Thomas, where infection is not known to exist, the authors were unable to find any reactors in the group examined.

The method follows: Large amounts of *Br. abortus* are grown on standard nutrient agar pH 7.8 in Blake bottles for seventy-two hours at 37° C. Approximately 5 ml. of solution with 0.5 per cent phenol are added to each bottle and left for thirty minutes in contact with the culture to loosen the cells. The phenolized saline containing the suspension of cells is removed from the bottles with sterile pipettes, and the surface of the agar is thoroughly

washed with more phenolized saline. The organisms are recovered by centrifuging at high speed, and dried in a calcium chloride desiccator until they attain a horny appearance. Then they are ground in an electric grinding apparatus to obtain a fine white powder. This fine powder is mixed with 2 liters of 0.02 N sodium carbonate and placed in the refrigerator overnight. Next morning the alkaline suspension is filtered, first through a paper and then through a Berkefeld candle. To the filtrate is added 0.5 per cent phenol, to avoid bacterial contamination during the following steps of the procedure. The filtrate is then concentrated by ultrafiltration through alundum cups impregnated with 13 per cent gum-cotton glacial acetic solution, prepared according to the method described by Seibert. The ultrafiltration is continued until the liquid is concentrated to about 100 ml., then it is washed by passing 500 ml. of distilled water through the alundum cup filter. The concentrated colloidal solution is then filtered through a paper to eliminate any insoluble material that may have sedimented during the ultrafiltration. The protein is precipitated by mixing the colloidal solution with one-fourth its volume of a 50 per cent freshly prepared aqueous solution of trichloroacetic acid, making a final concentration of 10 per cent acid. After standing overnight in the icebox, the protein precipitate is thrown down by centrifugation. Then it is washed repeatedly by centrifugation with freshly prepared 10 per cent trichloroacetic acid solution until the washings are colorless. The protein product is then washed with large volumes of anhydrous ether by repeated triturations and centrifugations until all the acid is removed and the precipitate is completely dehydrated. The nitrogen and polysaccharide contents of protein prepared from different batches of cell cultures vary between 1 and 2 per cent.

The final product thus obtained fulfills the requirements, for it is of a fine consistency, light brown in color, and of a fairly constant chemical composition; it can be measured accurately, is stable in the dry state, and constitutes a good antigen. It is not completely soluble in water, but is easily dissolved by adding a few drops of 0.1 N alkali. The solution is neutralized with 0.1 N hydrochloric acid and remains clear. This preparation has been used in determining cutaneous hypersensitiveness among contact groups and groups from the general population of the island of Puerto Rico, where endemic abortion is prevalent, and in the island of St. Thomas, where endemic abortion is not known to exist. At the same time, agglutinative, complement fixation, and opsonocytophagocytic tests were made on the same subjects.

GLUCOSE TOLERANCE, Effect of Large Doses of Insulin on, Appel, J. W., and Hughes, J. Am. J. M. Sc. 199: 829, 1940.

The authors recognize the great value of the glucose tolerance test as usually performed. However, the examination of the urine during the three days following the test appears to be even more useful in determining the actual carbohydrate tolerance. In the cases reported the usual tests would have indicated either more insulin or less carbohydrate, though further examinations as described showed that neither step was indicated.

Two of the cases examined over several weeks showed subjective and objective clinical improvement. Thus it would seem that the authors' procedure could be employed to advantage where more carbohydrate is desirable but more insulin is contraindicated. A simple glucose tolerance test without blood sugar estimations but with recordings of the twenty-four hour urinary glucose over a period of several days before and after the test will indicate whether a given case can utilize additional carbohydrate.

The authors believe that many diabetic persons would benefit from the procedure herein described.

TUBERCLE BACILLI, Effect of Egg Oil on, Steenken, W., Jr. Am. Rev. Tuberc. 42: 422, 1940.

The experiments reported demonstrate that the tubercle bacillus will propagate in egg oil and that the resulting colonies will be smooth and creamy. They also show that this smooth appearance will be manifested by both dissociated variants and by undissociated cultures, and that the resulting colonies cannot be differentiated from one another.

These smooth colonies retain their altered appearance only while they are growing in the presence of the egg oil. Since this occurs regardless of the pH of the medium, the process cannot be a true dissociation; the smooth form of the colony is merely a physical effect of the oil upon its contours.

BLOOD, A New Test for, and Its Application to the Micro-Determination of Hemoglobin, Barrett, J. F. Brit. J. Exper. Path. 21: 22, 1940.

The reduced form of the oxidation reduction indicator 2, 6-dichlorophenol indophenol is a useful reagent for the detection of blood. In the presence of hydrogen peroxide and blood pigment a red color is produced. A response is obtained with a 1:50,000 dilution of blood.

The reaction has been utilized to determine the hemoglobin content of minute quantities of blood. The equivalent of 0.00025 ml. is required for a determination.

The method for hemoglobin determination follows:

Reagents.—(a) 0.2 per cent alcoholic hydrogen peroxide. Add 0.5 ml. of 30 per cent hydrogen peroxide (perhydrol) to 75 ml. alcohol.

(b) Reduced indicator solution. Mix 0.2 Gm. of 2, 6-dichlorophenol indophenol with 120 ml. of glacial acetic acid. Add 25 ml. of 1 per cent stannous chloride solution ($\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$) in 20 per cent acetic acid. Stir until the dye dissolves, warming slightly if the dye is granular. Finally add 20 Gm. of crystalline sodium acetate dissolved in 50 ml. of water. Store the reagent in a dark stoppered bottle.

(c) Stock hemoglobin solution. Determine the hemoglobin content of a sample of normal blood by the oxygen capacity method of Van Slyke and Neill (1924). Dilute the blood with 25 per cent alcohol to give a solution of hemoglobin containing 1.3 Gm. per 100 ml. Store the solution in a stoppered bottle in the refrigerator.

Test for Blood.—Place 5 ml. of the specimen in a clean test tube, add 2 ml. of glacial acetic acid and boil the mixture thoroughly. Cool the tube to room temperature and add 5 ml. of ether. Extract the blood pigment by inverting the tube about twelve times. Place 5 to 10 drops of reduced indicator solution in a second test tube, and add 2 to 3 ml. of alcoholic hydrogen peroxide. Transfer 1 to 2 ml. of ethereal extract from the first test tube to the mixture of reduced dye and hydrogen peroxide in the second test tube. A rose-red color develops in the presence of blood. If no color appears in two minutes, the test is negative.

Microdetermination of Hemoglobin.—Dilute the well-mixed specimen of blood 2,000 times with water, using a carefully calibrated pipette. Prepare a working standard solution of hemoglobin by diluting the stock 1.3 per cent hemoglobin solution 200 times with water, e.g. 0.5 ml. to 100 ml.

Pipette 2 ml. of the reduced indicator solution into two test tubes labeled "standard" and "unknown." Add 2 ml. of the alcoholic hydrogen peroxide and mix thoroughly, making sure that no hydrogen peroxide is left on the sides. Add 0.5 ml. of the standard and immediately afterwards 0.5 ml. of the unknown solution of hemoglobin to the appropriate tubes and mix thoroughly. After one hour add 10 ml. of water to each tube, inverting several times to mix the contents. Compare the unknown with the standard in the colorimeter, using a green glass light filter to facilitate the matching.

Calculation: $\frac{\text{Reading of Standard}}{\text{Reading of Unknown}} \times 13 = \text{Grams of hemoglobin per 100 ml. blood}$

When a blood low in hemoglobin is encountered, a more suitable dilution, e.g. 1:1,000 or 1:500, should be made.

LEUKOCYTOSIS, On the Mechanism of, With Inflammation, Menkin, V. Am. J. Path. 16: 13, 1940.

Leukotaxine, a crystalline nitrogenous substance recovered from inflammatory exudates, and known to increase capillary permeability and cause diapedesis of polymorphonuclear leucocytes, fails to induce an increase in the leucocytic level of the circulation in either the dog or the rabbit. Hence it can scarcely be responsible for the leucocytosis of inflammation.

There is present in exudates a leucocytosis-promoting factor capable of inducing a rise in the leucocyte level of dogs. This is demonstrable when either the whole or the cell-free exudate is introduced into the circulating blood.

An inflammatory exudate removed from an animal with leucopenia and injected into a normal dog fails, as a rule, to induce a marked rise in the leucocyte count.

Blood serum, sterile broth, bacterial cultures of exudates, and cultures of killed bacteria (*Staphylococcus aureus*) are all ineffective in causing an increase in the leucocyte level of the circulation.

Histamine and adenosine are likewise essentially inactive. Extraction of exudates containing the active leucocytosis-promoting factor for histamine, yields an end product incapable of inducing leucocytosis. Nucleic acid injected into the blood stream in large concentration favors, after a considerable latent period, a rise in the number of leucocytes. This delayed effect differs from the prompt response induced by exudates.

The action of the leucocytosis-promoting factor seems to be primarily on the bone marrow, producing an outpouring of immature granulocytes into the circulation. The factor is thermolabile. Heating the exudate to 60° C. inactivates the material. The factor, after prolonged dialysis of the exudate through a cellophane membrane, is found to be, in large part, indiffusible. Further studies are now in progress in an endeavor to identify the nature of the leucocytosis-promoting factor present in inflammatory exudates.

PNEUMONIA in Infants and Children, Pneumococcus Typing in, Bullowa, J. G. M., and Simon, H. Am. J. Dis. Child. 60: 256, 1940.

With the method of pharyngeal evacuation applied to a mummied infant and accomplished by the use of a suction bulb with catheter and trap, pulmonary mucus obtained for typing pneumococci gave a type in 95 per cent of cases. The portable apparatus for evacuation is simple to prepare and use.

The apparatus consists of a No. 18 French rubber catheter, with an opening at the end. The catheter is attached to a fire-polished and annealed pyrex glass trap about 4 inches (10.16 cm.) long. From the glass trap through pressure rubber tubing, the catheter is attached by brass tubing to a sputum bottle stoppered with a No. 5 rubber stopper containing two holes. The outlet from the sputum bottle, also of brass, leads to a double action rubber bulb or to an electric suction pump. The pump is of the rotary compressor type, operated by a $\frac{1}{30}$ horse power universal motor.

INFECTION, A Ward Study for Sources of, Long, A. P., McKhann, C. F., and Cheney, L. L. Am. J. Dis. Child. 60: 322, 1940.

A direct, concurrent study of hospital infections in an infants' hospital confirmed the findings derived from an earlier survey of the records of the same institution.

Infections of the respiratory tract accounted for 63 per cent of the hospital infections which were acquired by over 10 per cent of the patients admitted. Most of the secondary infections occurred in very young, premature, malnourished, or debilitated infants who had been in the hospital for several weeks.

Acute infections of the respiratory tract, including pneumonia, present among the patients on admission as well as similar infections and carrier states among the staff, attendants, and visitors, were the great source of danger.

Examinations for new nasopharyngeal bacterial invaders in secondarily infected patients indicated the source of most of the infections to be hospital personnel and visitors rather than fellow patients.

Overcrowding in connection with hospital-acquired infections should be considered, with the term "overcrowding" applied not only to patients but also to the large numbers of persons other than patients who are permitted to enter the wards.

Some diminution in the transmission of organisms followed masking of all persons entering the ward. Masking, even with a special type of mask, was insufficient to eliminate secondary infections.

TISSUE: Rapid Method of Staining Gram-Positive Organisms in Frozen Sections
Krajian, A. Arch. Path. 30: 614, 1940.

Method.—

1. Fix tissues in a 10 per cent solution of formaldehyde for twenty-four hours or longer.
 2. Cut thin frozen sections.
 3. Transfer the section to a glass slide.
 4. Drain and dehydrate immediately with absolute alcohol or anhydrous isopropanol.
- Blow on the section until all the alcohol is evaporated.
5. Blot dry.
 6. Dip twice in thin celloidin (a pyroxylin preparation). Blow on the section and dip in water.
 7. Stain with Sterling's gentian violet for two minutes.
 8. Rinse in water and apply Gram's iodine solution for two minutes.
 9. Blot completely dry.
 10. Flood the section with eosinol (with medicine dropper) and agitate the slide. Generally one or two applications are sufficient to remove the gentian violet and at the same time counterstain the section red.
 11. Treat with carbolxylene for thirty seconds.
 12. Treat with two changes of pure xylene, fifteen seconds each.
 13. Mount in gum dammar.

Results: Gram-positive organisms (bacteria and fungi) are stained violet; gram-negative organisms are unstained; the background is bright red.

For emergency examination, fresh tissues are fixed as follows:

A 10 per cent formaldehyde solution is brought to a boil, and thin blocks of tissue are dropped into it. The container is placed in a paraffin oven at 56° to 60° C. for fifteen minutes' fixation. Then frozen sections are prepared and stained as already described.

Preparation of Eosinol.—Dissolve 5 Gm. of aqueous eosin in 10 c.c. of distilled water. Precipitate it by adding 10 c.c. of glacial acetic acid and 2 c.c. of concentrated hydrochloric acid, and mix with a glass rod.

Incubate the resulting coagulum at 56° C. for from twelve to sixteen hours, or until the water has evaporated.

Dissolve this dehydrated acid eosin in 10 c.c. of absolute alcohol or anhydrous isopropanol and 20 c.c. of acetone, stirring with a glass rod for several minutes and placing in a paraffin oven for two hours. Let the undissolved portion, which is to be discarded, settle to the bottom of the container. Remove the clear portion with a clean, dry pipette, add to 1,500 c.c. of carbolxylene (1 part of pure phenol crystals in 3 parts of neutral xylene). Some precipitate will form, which will settle to the bottom of the container. The clear portion is eosinol. The solution keeps indefinitely.

Owing to the variation in the staining power of various brands of powdered eosin, it may be necessary to standardize the solution by staining control sections and adjusting the strength of the eosinol by reducing or increasing the amount of carbolxylene.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor,
Dr. Warren T. Vnuglian, Professional Building, Richmond, Va.

Poisons, Their Isolation and Identification*

AS NOTED in the Foreword, the number of poisoning cases in Egypt is sufficiently large to engage permanently a force of chemists in the necessary medicolegal determinations. This book, written by the late Director of the Medicolegal Laboratory of the Ministry of Justice is, therefore, based upon an extensive and comprehensive experience.

Each procedure detailed has been personally used and found reliable by the author. The book is hence a record of practical experience and as such should prove of great value to others engaged in this field.

The author presents in Chapter X a systematic scheme for the identification of alkaloids embodying much that is original. A complete author index and a comprehensive general index make the contents of the book readily available.

Intended as a practical manual for all chemists who have to deal with poisoning cases, this book should fill a definite need.

The Complete Pediatrician†

THE necessity for a third edition in six years is ample testimony to the practical clinical utility of this book. Comprehensive and well organized, it embodies perhaps the most complete epitome in small compass of pediatrics yet available.

It can be consulted with confidence that the information sought will be found and that when found it will be in readily applicable form.

This new and revised edition should prove as welcome and as useful to the clinician as did its predecessors.

The Essentials of Applied Medical Laboratory Technic‡

A PROFUSELY illustrated volume describing the generally accepted routines in clinical laboratory work, this book should be of especial value for the clinical pathologist's assistant and can be very highly recommended to all technicians. Indeed, it will probably become a widely used textbook for technicians.

Embalming Fluids§

A COMPREHENSIVE discussion of the art of embalming from the earliest to the present time, this book contains much information of interest, not only to embalmers, but also to physicians and pathologists and all who are interested in the historical aspects of this subject.

*Poisons, Their Isolation and Identification. By Frank Bamford, B.S., late Director of Medicolegal Laboratory, Ministry of Justice, Cairo, Egypt. With Foreword by Sydney Smith, M.D., F.R.C.P. Washable binding, 344 pages, 21 illustrations, \$4.00. P. Blakiston's Son & Co., Philadelphia, Pa.

†The Complete Pediatrician. Practical, Diagnostic, Therapeutic, and Preventive Pediatrics. By W. C. Davison, M.D., Professor of Pediatrics, Duke University School of Medicine. Cloth, ed. 3, 256 pages, \$4.00. Duke University Press, Durham, N. C.

‡The Essentials of Applied Medical Laboratory Technic. Details of How to Build and Conduct a Laboratory in Hospital or Office at Small Cost. By J. M. Feder, M.D., Director of Laboratories and Allergic Service, Anderson County Hospital, Anderson, S. C., Cloth, 234 pages, profusely illustrated, two plates in color, \$5.00. Charlotte Medical Press, Charlotte, N. C., 1940.

§Embalming Fluids. Their Historical Development and Formulation From the Standpoint of the Chemical Aspects of the Scientific Art of Preserving Human Remains. By Simon Mendelsohn, F.A.I.C., Consulting Chemist. Cloth, 166 pages, 6 figures and frontispiece, \$4.00. The Chemical Publishing Co., New York, N. Y.

Histopathology of the Peripheral and Central Nervous Systems*

HERE is a volume that should always be available as a companion piece to any text on clinical neurology. Its function is similar to that of the texts of general pathology in relation to texts on general medicine. It is very complete, with discussion of very recent studies, including the many forms of meningitis and encephalitis and the myopathies.

The book is abundantly and well illustrated.

Laboratory Text in Pharmacology†

THIS excellent manual of experimental exercises is intended to serve as a supplement to the more complete texts. It was developed over the course of several years; hence it has been subjected to the vicissitudes of actual laboratory use.

Valuable tips are given as to the proper technique of administering drugs to various mammals. The reviewer can recall a ruined experiment which would have had better results had he been forewarned "that rabbits are very adept at kicking syringes and bottles off the table."

The second portion of the book deals with prescription writing and materia medica, the former of which gives evidence of becoming a lost art under the pressure of commercial preparations. Throughout, frequent references are given to the current literature.

The manual can be highly recommended.

Pathological Histology‡

THE student of pathology and the physician looking for a "refresher" course will find this a text of outstanding value for the purpose. The pathologist, too, will find it a valuable addition to his reference library.

The text is succinct and authoritative. The numerous illustrations—Fialay-process transparencies of excellent quality excellently reproduced—are well chosen and worthy of the highest commendation. They are, indeed, an outstanding feature of the book which can be highly recommended without reserve. Altogether an excellent "buy," it is moderately priced and well worth its cost.

Erratum

On page 730 of the January number of the JOURNAL in the article by Ida Kraus, Ph.D., and Sol Dulkan, B.S., entitled "The Determination of Hippuric Acid in Urine," the eleventh line of the paragraph below Table I should read "ten normal sulfuric acid" instead of "tenth normal sulfuric acid." On page 731 the seventh line of the second paragraph below Table II should read "10 normal sulfuric acid" instead of "1:1 sulfuric acid."

*Histopathology of the Peripheral and Central Nervous Systems. By George B. Hassin, M.D., Professor of Neurology, University of Illinois, College of Medicine, Attending Neurologist, Cook County Hospital, Chicago. Cloth, ed. 2. (revised and enlarged), 302 illustrations, 554 pages. \$7.50. Paul B. Hoeber, Inc., Medical Book Department of Harper & Brothers, New York, 1910.

†Laboratory Text in Pharmacology. By Robert P. Walton, Professor of Pharmacology, School of Medicine, University of Mississippi. Paper, 85 pages, \$1.50. J. B. Lippincott Company, Philadelphia, London, Montreal.

Robertson F. Olgivie, M.D., F.R.C.P. (Edin.), Lecturer in Pathology, Royal Infirmary, Edinburgh. Examiner in Pathology for the Triple Qualification, F.R.C.P. (Edin.), Professor of Pathology, F.R.C.P. (Edin.). A William Wood Book. Colored plates, \$8.50.

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CLINICAL AND EXPERIMENTAL

THE DIAGNOSTIC VALUE OF THE TAKATA-ARA REACTION IN THE CEREBROSPINAL FLUID*

PAUL B. SZANTO, M.D., AND SAMUEL BURACK, M.D., KANKAKEE, ILL.

LABORATORY tests devised for the examination of the cerebrospinal fluid are numerous. Despite this we agree with Kafka¹ that investigation of proteins in cerebrospinal fluid opens up new fields of research, particularly in the albumin-globulin relationship.

Eskuchen² stressed the importance of the different colloidal and protein reactions, the proteins (albumin and globulin) varying in their sensitivity to the various reagents. He introduced the term "protein spectrum." We must also consider the statement of Kafka³ that pathologic processes result not only in quantitative change of the globulin and albumin and change of the ratio between both, but also qualitative alteration of the proteins themselves.

In 1925 Takata and Ara⁴ introduced a new serum colloid test for differentiation between lobar and bronchopneumonia. In the same year they⁵ used the test for examination of the cerebrospinal fluid to differentiate between bacterial meningitis and syphilis of the central nervous system.

The reaction is based on the following: If a solution of mercuric chloride is mixed with sodium carbonate in the presence of a protein colloidal system, for example, spinal fluid, there results a colloidal solution (HgO). The addition of fuchsin solution results in the formation of a violet blue color because the fuchsin is adsorbed by the colloid (HgO). In syphilitic spinal fluid there occurs a precipitation of the colloids with a resulting violet blue to blue color. Spinal fluid of bacterial meningitis gives a red color with little or no precipitation. Normal spinal fluid gives a violet blue color with no precipitation.

In 1929 Staub⁶ and Jezler⁷ introduced the Takata-Ara serum test for the diagnosis of parenchymatous liver diseases, especially for the diagnosis of liver

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cirrrosis. In the following years many investigators (Staub,⁸ Skouge,⁹ Schindel and Barth,¹⁰ Takata,¹¹ Kirk,¹² and Fulde¹³) affirmed the diagnostic value of this test for hepatic cirrhosis.

The reports of Takata and Ara on the cerebrospinal fluid in central nervous system syphilis were confirmed for the most part by various workers.

Münzer¹⁴ examined 100 persons, of whom 30 had general paresis. These 30 gave the paretic type of Takata reaction, while the bacterial meningitis patients did not give consistent results. In those with paresis there was a parallelism between the Takata and Lange tests.

Nicole¹⁵ found the Takata test more sensitive than the Ross-Jones, Weichbrodt, and Nonne-Apelt tests. He stated that the test was not specific for bacterial meningitis. Blum,¹⁶ Cameron and McCulloch,¹⁷ and Monias¹⁸ reported similar findings.

Walton¹⁹ examined 350 spinal fluids and found a parallelism between the Takata test and the Lange and mastie tests. He also stated the test was not specific for bacterial meningitis. All the workers mentioned followed more or less the original technique of Takata and Ara. Ueko²⁰ modified the Takata blood serum test, and this modification was applied to the spinal fluid by Fleischhaecker.²¹ Ornstein used the same modification, and both he and Fleischhaecker affirmed the diagnostic value of the modified Takata-Ara reaction for neurosyphilis.

We felt it would be worth while to evaluate the results of the Takata-Ara test for the following reasons: First, we wished to prove the specificity of the test as originally described by Takata and Ara. Our material did not lend itself to evaluation of the specificity of the test for bacterial meningitis. Second, we wished to compare the diagnostic value of the test in relation to other routine cerebrospinal fluid determinations. Third, we wished to see which modification gave best results from the standpoint of both simplicity and sensitivity. Finally, we attempted to reach a better understanding of the mechanism behind the test.

TECHNIQUE AND INTERPRETATION

The Takata-Ara reaction can be performed in two ways: (1) the original five-tube method and (2) the so-called one-tube method. In the original technique, 1 c.c. of 0.3 per cent sodium chloride solution is added to four of five Wassermann test tubes. One cubic centimeter of spinal fluid is placed in the first and second tubes. The second tube is well agitated, and 1 c.c. of the mixture is added to the third tube, etc. One cubic centimeter from the fifth tube is discarded. One drop of 10 per cent sodium carbonate solution is added to each tube, followed by 0.3 c.c. of the Takata reagent. The Takata reagent consists of 0.5 per cent mercuric chloride and 0.02 per cent fuchsine solution in equal amounts. The tubes are well shaken and left at room temperature. The results are read after five, fifteen, and thirty minutes, and again after twenty-four hours. If the precipitation occurs immediately, or within a few minutes, we have a three-plus reaction; if the precipitation is complete after fifteen minutes, the reaction is two plus; after thirty minutes, one plus; and after twenty-four hours, one plus. The results were plotted on a curve. Spinal fluid from neurosyphilis gives complete precipitation in a short time.

In the one-tube method Ueko's modification was applied, wherein fuchsin solution is not used. The reagents required are 10 per cent anhydrous sodium carbonate and 0.5 per cent mercuric chloride solution. One cubic centimeter of spinal fluid is placed in a Wassermann tube to which is added one drop of the sodium carbonate solution and 0.1 c.c. of mercuric chloride solution. The exact amount of sodium carbonate used is very important because this substance influences the pH of the entire colloidal system. Glasoe and Sorum²² showed that the sensitivity of a gold sol in the Lange spinal fluid test for paresis increases with an increase in the particle size, and decreases with an increase in the pH. We were able to show that the same principle applies to the Takata-Ara test. The results are read immediately, then after five, fifteen, thirty, sixty, and ninety minutes.

The interpretation of the results in the one-tube method is as follows: Three plus is indicated when flocculation occurs immediately with sedimentation. In a two-plus reaction complete flocculation and sedimentation occur in from five to thirty minutes. A one-plus reaction is indicated when an opacity occurs within thirty minutes. The reaction is negative when the mixture remains clear, or when there is a very slight opacity after thirty minutes.

At first we used both methods. However, we soon saw that our results with the one-tube method were as accurate as with the five-tube method.

MATERIAL AND RESULTS

We examined 314 persons. Of these there were 167 with general paresis, treated and untreated. Many of the treated patients had completely negative routine laboratory findings. In addition we had 147 individuals without paresis, 54 with dementia praecox, 40 with chronic alcoholism, 8 with idiopathic epilepsy, 9 with mental deficiency, 4 with psychoneurosis, 1 with pachymeningitis hemorrhagica interna, 1 with multiple sclerosis, 2 with manic-depressive psychosis, 15 with cerebral arteriosclerosis, 12 without psychosis, and 1 with traumatic psychosis.

GENERAL PARESIS

Our group of patients with general paresis had been on treatment for a number of years and some of them had resultant negative serologic and spinal fluid findings.

Of the 167 cases there were 34 with three-plus Takata reaction. In every case the spinal Wassermann was strongly positive. The Ross-Jones test gave a positive result in 19 cases, a trace in 10, and a negative result in 5. The cell count was less than ten in 11 cases, between ten and fifty in 14, and over fifty in 9. The gold sol reaction was in the first zone in 32 cases, and in the middle zone in 2.

There were 13 cases of two-plus Takata reaction. The spinal Wassermann was positive in every case. The Ross-Jones test was positive in 4, showed a trace in 7, and was negative in 2. The cell count was less than ten in 4 cases, between ten and fifty in 7, and over fifty in 2. The gold sol reaction was in the first zone in 7 cases, and in the middle zone in 6.

Sixty-seven persons gave a one-plus Takata reaction. The spinal Wassermann was positive in 64 and negative in 3. The Ross-Jones test was positive

in 2, showed a trace in 23, and was negative in 42. The cell count was less than ten in 49, between ten and fifty in 15, and over fifty in 3. The gold sol reaction was in the first zone in 1 case, in the middle zone in 32, in the end zone in 32, and negative in 2 cases.

There were 53 persons with negative Takata reaction. The spinal Wassermann was positive in 13 and negative in 40. The Ross-Jones test showed a trace in 2, and was negative in 51. The cell count was less than ten in 52 cases and between ten and fifty in 1. The gold sol reaction was in the first zone in 1 case and in the end zone in 3; there was none in the middle zone, and 49 cases were negative.

In the 147 persons without paresis the Takata reaction was negative.

The Takata-Ara reaction resembles the Weichbrodt test in that it utilizes mercuric chloride. However, there is a striking difference in the results. In the Weichbrodt test 0.3 c.c. of 1:2,000 mercuric chloride solution is added to 0.7 c.c. of spinal fluid in a test tube. A positive test is indicated by flocculation or opacity. We performed this test in 49 patients. In 31 both Takata-Ara and Weichbrodt tests were negative; in 18 the Takata-Ara reaction was positive and the Weichbrodt test was negative.

TABLE I

RELATIONSHIP OF TAKATA-ARA AND GOLD SOL REACTIONS TO THE GLOBULIN-ALBUMIN RATIO

CASE	SPINAL WASSER- MANN	LANGE	CELL COUNT	ROSS- JONES	TAKATA	GLOBULIN ALBUMIN
W. H.	++++	5555433111	62	Positive	+++	1.5
M. B.	++++	5444321100	65	Positive	+++	2.2
R. D.	++++	5555432111	66	Positive	+++	2.6
W. B.	++++	3342211100	42	Positive	++	1.4
G. W.	++++	5543311110	48	Positive	+++	1.9
V. M.	++++ in 1 c.c.; nega- tive in 0.5 c.c.	2221110000	11	Negative	+	1.1
M. D.	++++	4332111000	6	Negative	+++	1.7
G. W.	++++	4332211000	15	Trace	++	1.5
E. F.	++++	5432111000	29	Trace	+++	2.8
T. D.	++++	5544432111	266	Positive	+++	2.4
T. M.	++++	5554331110	34	Trace	++	1.6
G. Y.	++++	3221110000	67	Trace	+	1.2
L. B.	++++	5433211100	28	Positive	+++	2.4
O. H.	++++	5543321110	40	Trace	+++	2.2
R. R.	++++	5554432110	21	Negative	+++	2.5
G. P.	++++	5554443210	36	Positive	+++	2.0

COMMENT

Our results indicate a striking parallelism between the Takata reaction and the gold sol reaction. Of the 114 persons with general paresis with positive Takata reaction the gold sol reaction was negative in only two. Of the 53 persons with general paresis with negative Takata reaction the gold sol reaction was positive in only 4. In addition, the degree of the Takata reaction shows a parallelism with the gold sol curve. The Takata reaction is much more sensitive than the Ross-Jones test. Of the 114 persons with positive Takata reaction the Ross-Jones test was negative in 49 cases, or 44 per cent. In regard to the cell count there is no parallelism.

15. Nicole, R.: Die Takata-Ara Reaktion in Liquor Cerebrospinalis, *Ztschr. f. klin. Med.* 106: 192, 1927.
16. Blum, E.: Nachprüfung der kolloidehemischen Liquorreaktion von Takata-Ara an 250 Fällen, *Ztschr. f. d. ges. Neurol. u. Psychiat.* 110: 504, 1927.
17. Cameron, D. E., and McCulloch, R.: Takata-Ara Test for Cerebrospinal Fluid, *Canad. M. A. J.* 23: 557, 1930.
18. Monias, B. L.: Clinical Value of Spinal Fluid Test of Takata and Ara, *J. LAB. & CLIN. MED.* 14: 67, 1928.
19. Walton, S. T.: A Comparative Study of 350 Spinal Fluid Examinations With Takata-Ara Test, *J. LAB. & CLIN. MED.* 16: 1125, 1931.
20. Ucko, H.: (a) Serum Test for Diagnosis of Liver Disturbances, *Guy's Hosp. Rep.* 88: 166, 1936; (b) Réaction de précipitation par le mercure dans le serum des hépatiques, *Compt. rend. Soc. de biol.* 118: 534, 1935.
21. Fleischhacker, H. H.: The Takata Reaction on the Cerebrospinal Fluid in Syphilis of the Nervous System, *J. Ment. Sc.* 84: 378, 1938.
22. Glasoe, P. U., and Sorum, C. M.: The Lange Test, *J. LAB. & CLIN. MED.* 25: 1, 1933.
23. (1) Kafka, V., and Samson, K.: Die Eiweissrelation des Liquor cerebrospinalis, (a) III. Mitteilung, *Ztschr. f. d. ges. Neurol. u. Psychiat.* 117: 128, 1928; (b) IV. Mitteilung, *Ibid.* 119: 153, 1929; (c) V. Mitteilung, *Ibid.* 120: 744, 1929. (2) Kafka, V.: Die Cerebrospinal Flüssigkeit, Leipzig and Vienna, 1930, F. Deuticke.

100 EAST JEFFERY STREET

EPINEPHRINE SECRETION FROM THE ADRENAL GLANDS IN RELATION TO PARATHYROID ACTIVITY*

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IN AN earlier study¹ it was shown that the rate of liberation of epinephrine from the adrenal glands in dogs with experimental hyperthyroidism is not different from that in normal, control animals. Experiments on hypothyroidism, induced by extirpation of the thyroids, may be complicated by possible effects of concurrent hypoparathyroidism. Experiments on thyroparathyroidectomized animals, therefore, have been included in this report.

Probability of functional interrelationship between the parathyroids and the adrenals is suggested from certain earlier observations.² The evidence indicates that such an interrelationship is probably concerned with the function of the adrenal cortex, but it does not exclude the possibility that the medulla may play a significant role in the phenomena observed.

Rogoff and Stewart³ found that adrenalectomized dogs develop symptoms that are commonly associated with disturbances in parathyroid function. Muscular twitching, spasms, and tetanic convulsions often occur. Furthermore, bilateral adrenalectomy is commonly followed by an increase in calcium content of the blood.⁴ At autopsy severe congestion and hemorrhages are found in the alimentary canal of adrenalectomized dogs, similar to that observed by Collip⁵ in unoperated dogs following administration of large amounts of parathyroid extract.

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The changes that occur in the blood and the disturbances in the alimentary canal that are found during acute adrenal insufficiency in adrenalectomized dogs have been observed in animals with subacute and chronic adrenal insufficiency created by the method described by Rogoff.⁶ In these animals, as well as in those surviving bilateral adrenalectomy without treatment nearly or up to a fortnight, it has been observed that there is a relatively high incidence of parathyroid enlargement.² Some of the blood changes that occur in these animals are the same as those caused by administration of parathyroid extract.

Certain changes that occur in the rat incisor following bilateral adrenalectomy were found by Schour and Rogoff⁷ to be similar to the disturbances in calcification of the dentine observed by Schour, Tweedy, and McJunkin⁸ following administration of parathyroid extract. The rat incisor as an indicator for changes in calcium metabolism is suggested from the sensitive response to parathyroidectomy observed by Erdheim.⁹

The foregoing experimental evidence points to a probable adrenal-parathyroid interrelationship. It appears that the adrenal cortex is concerned with this possible interrelationship. Experimental data concerning the adrenal medulla in relation to parathyroid function is less convincing. Some observations on the pharmacodynamics of epinephrine suggest a possible influence on calcium metabolism. However, unless such observations are quantitatively related to amounts of epinephrine that the adrenals are known to be capable of liberating, they may be misleading. Mathien and Baer¹⁰ failed to obtain a significant effect on the blood calcium of dogs with parathyroid insufficiency when they administered physiologic quantities of epinephrine.

Some investigators have found a reduction in the epinephrine content of the adrenals in animals with parathyroid insufficiency,^{11, 12} while others observed no change.¹³⁻¹⁵ Of course, the amount of epinephrine stored in the adrenal cannot indicate the rate of its liberation; it can represent no more than the balance between production and liberation. However, these conflicting results suggest the possibility that thyroparathyroidectomy sometimes may lead to disturbances which interfere with storage of epinephrine in the adrenals. These disturbances may not develop in all thyroparathyroidectomized animals. This suggestion might be offered in explanation of similar observations regarding the rate of epinephrine secretion reported in this article.

Indirect evidence has been offered in support of the view that certain manifestations of parathyroid insufficiency are associated with a corresponding increase in circulating epinephrine.¹⁶ Czarnecki and Sarabia¹⁷ found no evidence of increased liberation of epinephrine from the adrenals in dogs with parathyroid insufficiency, and Georgopolous obtained negative results with peripheral blood of rabbits. These investigators did not make quantitative determinations of the rate of output of epinephrine from the adrenals.

Our investigation is concerned with the question whether experimental hypoparathyroidism or hyperparathyroidism is associated with a detectable change in the rate of liberation of epinephrine from the adrenal glands. Hyperparathyroidism was induced by administration of parathyroid extract,* and

*Generously supplied by Eli Lilly and Company.

hypoparathyroidism by thyroparathyroidectomy which, of course, includes any possible influence that might result from loss of the thyroid.

The characteristic manifestations of hypothyroidism are not readily obtained in adult dogs following thyroidectomy. This is generally attributed to the presence of accessory thyroid tissue or to incomplete removal of the thyroids. However, definite manifestations of hypothyroidism can be induced by complete thyroidectomy in young dogs if sufficient parathyroid tissue remains to prevent the complications of parathyroid deficiency. It may be assumed also that complete thyroidectomy in older dogs might be associated with some physiologic disturbances that are not as yet recognized.

TABLE I
EPINEPHRINE OUTPUT IN THYROPARATHYROIDECTOMIZED DOGS

RECORD NUMBER	SEX	BODY WEIGHT (KG.)		SERUM CALCIUM PER 100 C.C. (MG.)		ADRENAL WEIGHT (GM.)		EPINEPHRINE OUTPUT PER MINUTE (MG.)	
		INITIAL	FINAL	INITIAL	FINAL	RIGHT	LEFT	PER ANIMAL	PER KG.
21	M	11.7	10.1			0.73	0.76	0.00016	0.00016
38	M	12.0	12.0	10.17	6.10	0.52	0.57	0.0021	0.00017
61	M	8.8	8.5	11.85	7.44	0.40	0.40	0.00111	0.00013
62	M	7.3	6.3	12.91	4.30	0.41	0.38	0.00563	0.0009
68	F	9.8	8.8	10.02	4.03	0.64	0.64	0.0004	0.000045
69	M	10.5	10.7	10.60	5.55	0.62	0.59	0.00225	0.00021
71	M	7.3	7.5	11.66	5.28	0.49	0.50	0.00135	0.00018
73	M	13.0	11.9	12.27	4.88	0.70	0.70	0.0024	0.0002
74	F	8.0	7.6	10.88	5.76	0.56	0.56	0.00023	0.00003
2-15	M	12.1	11.5			0.78	0.76	0.0017	0.00015
1-81	M	13.3	13.5			0.85	0.76	0.0023	0.00017

TABLE II
EPINEPHRINE OUTPUT IN THYROPARATHYROIDECTOMIZED CATS

RECORD NUMBER	SEX	BODY WEIGHT (KG.)		DURATION (DAYS)	SYMPTOMS	ADRENAL WEIGHT (GM.)		EPINEPHRINE OUTPUT PER MINUTE (MG.)	
		INITIAL	FINAL			RIGHT	LEFT	PER ANIMAL	PER KG.
1-83	F	2.1	2.2	8	-	0.105	0.110	0.0004	0.00018
1-84	F	2.5	2.5	4	-	0.15	0.15	0.0002	0.00005
1-86	F	2.7	2.6	9	+-	0.21	0.20	0.00075	0.00029
1-96	F	1.9	1.9	11	-	0.08	0.09	0.00015	0.00008
2-22	M	4.54	3.9	14	+-	0.28	0.30	0.0011	0.00025
2-31	F	2.61	2.04	16	++	0.26	0.26	0.0002	0.0001
2-32	F	1.93	1.7	2	+++	0.15	0.18	0.0005	0.00029
2-33	F	3.64	3.3	2	++	0.33	0.33	0.00033	0.0001
2-34	F	2.73	2.27	6	+++	0.21	0.21	0.00025	0.00011
2-40	M	2.94	2.32	27	+-	0.14	0.12	0.0005	0.00022
2-41	F	3.18	3.63	50	+-	0.19	0.21	0.00025	0.00007

THYROPARATHYROIDECTOMY

Thyroparathyroidectomy was performed on 11 dogs (Table I) and 11 cats (Table II). Quantitative determination of the rate of liberation of epinephrine from the adrenals was made by the method of Stewart and Rogoff.¹⁵ The epinephrine output from the adrenals was determined at periods ranging between two and thirty days after thyroparathyroidectomy in the dogs, and between two and fifty days in the cats.

In the cat (2-41) on which determination of the epinephrine output was made fifty days after thyroparathyroidectomy, moderate symptoms were present during about half of the experimental period. The symptoms then gradually subsided. One cat (2-33) was thyroparathyroidectomized during advanced pregnancy. Marked tetany developed on the second day after the operation, indicating that the parathyroids of the fetuses did not protect the mother. That fetal endocrine organs do not function in place of the corresponding maternal organs when extirpated was found in the case of the adrenal (Stewart and Rogoff) and in recent observations (unpublished) in case of the pancreas.

Manifestations of parathyroid deficiency, ranging from apathy and mild muscular twitchings to severe convulsive seizures, were present in nearly all animals at the time when they were sacrificed for determination of the epinephrine output. In 8 of the dogs total calcium was determined in the blood serum by the Clark and Collip modification of the Kramer-Tisdall method.¹⁹ As shown in Table I, a decided reduction in serum calcium occurred in all animals. Some of the dogs received parathyroid extract or calcium therapy to control severe symptoms. In these cases the therapy was discontinued to permit the symptoms to reappear before the animal was used for determination of the epinephrine output. In the cats and in four of the dogs no therapeutic measures were employed.

Dog 21 developed symptoms of parathyroid insufficiency eight days after thyroparathyroidectomy. Calcium lactate (10 Gm. daily) was administered for eighteen days thereafter and was discontinued four days prior to determination of the epinephrine output. The symptoms were controlled by the calcium therapy and reappeared when therapy was discontinued. Clonic convulsions were present when the animal was sacrificed for determination of the epinephrine output.

In Dog 69 tetany developed on the fourth day after thyroparathyroidectomy. Calcium gluconate (10 c.c. of 10 per cent solution), which controlled the symptoms, was administered intravenously. Three days later the symptoms reappeared and the animal was sacrificed the following day for determination of the epinephrine output, without receiving any further treatment.

Dogs 61 and 62, both young, developed tetany on the second day following thyroparathyroidectomy. Each dog received 50 units of parathyroid extract. The symptoms were controlled, and Dog 61 was sacrificed the following day for determination of the epinephrine output. Dog 62 developed violent convulsions on the fifth day, and 30 units of parathyroid extract was administered. Determination of the epinephrine output was made the following day.

Dog 71 had tetany on the second day after thyroparathyroidectomy. He received 100 units of parathyroid extract and the symptoms were relieved. Four days later, when the symptoms were again in evidence, the epinephrine output from the adrenals was determined.

Dogs 38 and 74 developed severe tetany on the third and second day, respectively, following thyroparathyroidectomy. Determination of the epinephrine output from the adrenals was then made, without resorting to therapeutic measures.

Dog 68 developed mild tetany on the fourth day and it was present on the sixth day when determination of the epinephrine output was made. Mild tetany developed in Dog 73 on the fifth day following thyroparathyroidectomy, when the animal was sacrificed for determination of the epinephrine output from the adrenals.

HYPERPARATHYROIDISM

Ten dogs were subjected to the influence of parathyroid extract for periods ranging between one and fourteen days (Table III). In all cases a marked

elevation of the level of total calcium in the blood existed at the time when the animals were sacrificed for determination of the epinephrine output from the adrenals.

TABLE III

EPINEPHRINE OUTPUT IN DOGS WITH EXPERIMENTAL HYPERPARATHYROIDISM

RECORD NUMBER	SEX	BODY WEIGHT (KG.)		SERUM CALCIUM PER 100 C.C. (MG.)		COMBINED WEIGHT OF THYROID (GM.)	ADRENAL WEIGHT (GM.)		EPINEPHRINE OUTPUT PER MINUTE (MG.)	
		INITIAL	FINAL	INITIAL	FINAL		RIGHT	LEFT	PER ANIMAL	PER KG.
41	M	8.6	8.4	9.56	14.74		0.41	0.43	0.00227	0.00027
43	M	6.8	6.0	9.84	15.83		0.44	0.39	0.00024	0.00004
44	M	8.6	7.7	11.44	15.37		0.73	0.63	0.00078	0.0001
47	F	5.9	5.5	11.03	16.66	1.21	0.40	0.40	0.00067	0.00012
48	F	5.4	4.3	11.05	16.63	0.44	0.38	0.38	0.00146	0.00034
50	M	10.8	10.6	10.43	10.54	1.08	0.70	0.78	0.00233	0.00022
55	M	9.1	7.7	9.88	15.29	1.74	0.43	0.49	0.00185	0.00024
56	M	11.0	9.9	10.69	18.38	1.14	0.63	0.54	0.00109	0.00011
57	M	10.7	9.7	9.31	11.71	1.13	0.82	0.65	0.00262	0.00027
60	M	7.8	7.3	10.25	19.03	0.90	0.70	0.70	0.00204	0.00025

Dog 41 received 300 units of parathyroid extract in the morning and 200 units in the afternoon of the same day. Epinephrine output was determined eighteen hours after the last injection of parathyroid extract.

Dog 43 received 220 units of parathyroid extract on the first day, 250 units the next day, and 100 units on the third day. Epinephrine output was determined eighteen hours after the last injection of parathyroid extract.

Dog 44 received 200 units of parathyroid extract in the morning and 200 units in the afternoon of the same day. Epinephrine output was determined seventeen hours after the last injection of parathyroid extract.

Dog 47 received a daily injection of 100 units of parathyroid extract on each of three successive days. Epinephrine output was determined on the fourth day. Parathyroid extract caused considerable vomiting. Severe congestion and hemorrhage in the alimentary canal were found at autopsy.

Dog 48 received 50, 30, 30, 50, and 30 units of parathyroid extract on five consecutive days, respectively. Epinephrine output was determined on the sixth day.

Dog 50 received 50, 50, 50, 100, 100, and 100 units of parathyroid extract on six consecutive days, respectively. Epinephrine output was determined forty-eight hours after the last injection of extract.

Dog 55 received 50 units of parathyroid extract daily except on the sixth and twelfth days. On the thirteenth day 75 units were administered and the epinephrine output was determined on the fourteenth day.

Dog 56 received 50 units of parathyroid extract daily for twelve consecutive days. Epinephrine output was determined on the thirteenth day.

Dog 57 received 50 units of parathyroid extract daily for five consecutive days and 20 units on the sixth day. Epinephrine output was determined forty-eight hours after the last injection of parathyroid extract. A hemorrhagic ulcer was found in the stomach at autopsy. In this animal and in Dog 50 the serum calcium had returned to normal level when epinephrine output was determined.

Dog 60 received two injections of 50 units each of parathyroid extract, seven hours apart, on one day, and 50 units the next day; no parathyroid extract was administered the following four days; then 5 doses of 50 units each were administered during the next thirty hours. Epinephrine output was determined fifteen hours after the last injection of parathyroid extract.

DISCUSSION

In only one animal receiving parathyroid extract (Dog 43) was the rate of liberation of epinephrine from the adrenals found to be outside the range ordinarily occurring in normal animals. In view of the normal output found in the other nine animals, we hesitate to attach significance to the relatively low epinephrine output found in this animal.

None of the thyroparathyroidectomized cats showed significant alteration in the rate of liberation of epinephrine from the adrenals. Among the dogs, however, we found a definite reduction in epinephrine output in three of the eleven experimental animals. Normal rate of liberation was found in the other eight dogs. It appears probable that in some thyroparathyroidectomized dogs conditions may develop which can affect the adrenal medulla. This possibility might explain the conflicting results that have been reported concerning the epinephrine store in the adrenals, previously mentioned.

It is known that existence of accessory parathyroids is not uncommon. The suggestion may be made that such accessories, though functionally inadequate to prevent the usual manifestations of parathyroid deficiency in thyroparathyroidectomized animals, might suffice to prevent reduction of the epinephrine output. This could explain the negative results in the cats and in the 8 dogs that showed normal epinephrine output.

The possible influence of relative deficiency of thyroid, together with parathyroid deficiency occurring in some animals but not in others, may be suggested. In the light of present knowledge, however, this can be no more than mere speculation.

The results of our experiments, while not conclusive, support the existing suggestion of a probable functional interrelationship between the parathyroids and the adrenal medulla. Further investigation is necessary to obtain more unequivocal evidence. However, it appears that under some conditions, the exact nature of which is not known, parathyroid insufficiency can lead to a reduced rate of liberation of epinephrine from the adrenal glands.

SUMMARY

1. Under certain conditions, the nature of which is obscure, parathyroid insufficiency can lead to reduced epinephrine output from the adrenals.
2. Hyperparathyroidism, induced by administration of parathyroid extract, does not appear to influence the rate of epinephrine secretion from the adrenals.

REFERENCES

1. Rogoff, J. M., and Cortell, R.: Epinephrine Output From the Adrenal Glands in Experimental Hyperthyroidism, *J. LAB. & CLIN. MED.* 26: 981, 1941.
2. Rogoff, J. M.: Observations on Functional Interrelationship Between the Adrenal and Parathyroid Glands, *Science* 80: 319, 1934.
3. Rogoff, J. M., and Stewart, G. N.: Studies on Adrenal Insufficiency in Dogs. I. Control Animals Not Subjected to Any Treatment, *Am. J. Physiol.* 78: 683, 1926.
4. Rogoff, J. M., and Stewart, G. N.: Studies on Adrenal Insufficiency. VII. Further Blood Studies (Cholesterol and Calcium) in Control Adrenalectomized Dogs, *Am. J. Physiol.* 86: 25, 1928.
5. Collip, J. K.: The Production of Some of the Phenomena Peculiar to Parathyroid Overdosage in Dogs by Means of Certain Inorganic Salts, *Am. J. Physiol.* 76: 472, 1926.
6. Rogoff, J. M.: Experimental Production of Chronic and Subacute Adrenal Insufficiency in Dogs and Cats, *Proc. Soc. Exper. Biol. & Med.* 24: 1240, 1932.

7. Schour, I., and Rogoff, J. M.: The Influence of the Adrenal Glands on Calcium Metabolism, *Science* 83: 267, 1936; Changes in the Rat Incisor Following Bilateral Adrenalectomy, *Am. J. Physiol.* 115: 334, 1936.
8. Schour, I., Tweedy, W. R., and McJunkin, F. A.: The Effect of Single and Multiple Doses of the Parathyroid Hormone on the Calcification of the Dentine of the Rat Incisor, *Am. J. Path.* 10: 321, 1934.
9. Erdheim, J.: Tetania parathyreopriva, *Mitt. a. d. Grenzgeb. d. Med. u. Chir.* 16: 632, 1906.
10. Mathieu, F., and Bacq, Z. M.: Action of Epinephrine in Dog With Parathyroid Insufficiency, *Arch. internat. de physiol.* 38: 160, 1934.
11. Herring, P. T.: The Effects of Thyroidectomy and Thyroid-Feeding Upon the Adrenalin Content of the Suprarenals, *Quart. J. Exper. Physiol.* 9: 391, 1916.
12. Binet, L., and Weller, G.: La teneur des glandes surrénales en adrénaline chez les animaux parathyroprives, *Compt. rend. Soc. de biol.* 114: 985, 1933.
13. Gley, E.: Contribution à l'étude des interrelations humorales. II. Valeur physiologique de la glande surrénale des animaux éthyroïdes, *Arch. internat. de physiol.* 14: 175, 1914.
14. Bacq, Z. M., and Mathieu, F.: Teneur des glandes surrénales en adrénaline chez le chien en tétanie latente, *Arch. internat. de physiol.* 42: 279, 1935.
15. Georgopolous, M.: Beziehungen zwischen Parathyroidea und Chromaffinen System, *Ztschr. f. klin. Med.* 75: 411, 1912.
16. Falta, W., and Rudinger, C.: Klinische und experimentelle Studien über Tetanie, *Verhandl. d. 26 Kong. f. inn. Med.*, p. 405, 1909.
17. Czarnecki, E., and Sarabin, L.: La Sécretion surrénale d'adrénaline chez les Chiens éthyroïdes, *Compt. rend. Soc. de biol.* 97: 455, 1927.
18. Stewart, G. N., and Rogoff, J. M.: The Average Epinephrine Output in Cats and Dogs, *Am. J. Physiol.* 66: 235, 1923.
19. Clark, E. P., and Collip, J. B.: A Study of the Tisdall Method for the Determination of Blood Serum Calcium With a Suggested Modification, *J. Biol. Chem.* 63: 461, 1925.

A CASE OF INSULIN ALLERGY SIMULATING CORONARY OCCLUSION*

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NUMEROUS cases of insulin allergy have been reported in the literature. In the majority of these cases, local or generalized urticaria and angio-neurotic edema dominated the clinical picture. In a smaller number of instances, there were more severe allergic reactions, such as circulatory impairment, gastrointestinal symptoms, pulmonary edema, and collapse. Immunologic studies revealed that the observed hypersensitivity was due either to protein impurities accompanying the insulin or to pure crystalline insulin itself. Passive transfer of the sensitivity was effected in several instances. A comprehensive review of the literature has recently been published by Herzstein and Pollack.¹

The following case of insulin allergy, which followed the general immunologic pattern, presented an interesting, and as far as we know, a not yet described differential diagnostic problem.

A. J., a 65-year-old white woman, was admitted to the Lenox Hill Hospital for the first time on January 6, 1932, for the reduction and immobilization of a fracture of the left radius. She was found to be suffering from diabetes mellitus and was put on a diet and 20 units of insulin (Nletin) daily. She discontinued her diet after one year, but continued to take the 20 units of Nletin.

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She was readmitted to the hospital on July 21, 1936, for an osteomyelitis of the right toe. During her stay at the hospital she was again put on a diet and 20 units of Iletin. She was discharged on September 21, 1936, and told to discontinue her insulin as the diet alone sufficed to control the diabetes. The patient took no insulin until December, 1938, when 5 units were administered because of glycosuria and pruritus vulvae. Ten minutes after this injection she became unconscious, dyspneic, thrashed about, and had a cold sweat. She was given orange juice and completely recovered from the attack in about two hours. Since this episode she has been fearful of taking insulin.

Her third admission to the Lenox Hill Hospital was on April 3, 1939, for an ulcer of the right foot. Physical examination revealed a markedly obese elderly woman who did not appear acutely ill. Her temperature was 100.8° F.; her respirations were 20 per minute. There was no dyspnea or cyanosis. Numerous areas of vitiligo were present in the skin, and on the sole of the right foot was an ulcerated callus.

The pupils were equal, regular, and reacted to light and accommodation. The sclerae were clear. There was no exophthalmos, lagophthalmos, or nystagmus. The skin of the eyelids was loose, redundant, and had a puffy appearance. The conjunctivae were injected.

The mouth was edentulous. The tongue was slightly coated. The tonsils were small and cryptic, and the pharynx was congested.

The lungs were resonant throughout with normal breath sounds. No râles were audible.

The point of maximal impulse of the apex was in the fourth interspace in the mid-clavicular line. The border could not be accurately elicited because of obesity. The second aortic sound was accentuated and somewhat harsh. A systolic murmur was heard over the aortic area and a soft, low-pitched systolic murmur at the apex. The pulses were equal, regular, and the rate was 88. The blood pressure was 170 systolic and 90 diastolic.

No organs or masses were palpable in the abdomen. There was slight ankle and pretibial edema. The dorsalis pedis arterial pulsations were normal bilaterally. The reflexes were equal and active. There were no pathologic reflexes.

COURSE

The fasting blood sugar was 210 mg. per cent, and the urine revealed 1.4 per cent sugar and a trace of acetone. On April 5, 1939, the patient was given 20 units of Iletin in the morning after breakfast. Forty-five minutes after the injection the patient suddenly went into collapse. She complained of dizziness, vomited her breakfast, and was very dyspneic. There was no precordial pain. The skin was pale, cold, and clammy. The heart sounds were feeble and tic-tac in quality. The pulse was imperceptible at the wrist, and the blood pressure could not be obtained. Her venous pressure was 124 mm. The white blood count was 16,000; polymorphonuclear cells 81 per cent, twelve of which were immature; lymphocytes 18 per cent; monocytes 1 per cent.

An electrocardiogram taken fifteen minutes after the onset of the attack showed only minimal changes. These consisted principally of a depression of the S-T interval in Lead I, deep S and an inversion of the T-wave in Lead III, and a small R-wave and flattened T-wave in several of the precordial leads.

The patient was given 200 c.c. of orange juice and one-sixth grain of morphine, and was placed in an oxygen tent. She recovered rapidly and progressively from her shock; twelve hours later her blood pressure was 150 systolic and 100 diastolic; her heart sounds were of good quality and her pulse rate was 84.

Although the cardiologist considered various other possibilities, because of the white count of 16,000, the electrocardiographic changes, the fall in the blood pressure, and the tic-tac heart sounds, he favored the diagnosis of coronary occlusion and advised treating the patient for such. The features which militated against that diagnosis were the rapid recovery from the profound shock, the absence of temperature, and the normal erythrocyte sedimentation rate. In addition, a history was subsequently obtained of a similar attack three years previously following the injection of insulin.

During the next few days her blood pressure fluctuated between 90 systolic and 60 diastolic to 150 systolic and 90 diastolic. On April 6, the fasting blood sugar was 192 mg. per cent. The urine remained sugar free on diet alone, with only occasional slight spilling. The temperature remained normal throughout her stay. There was no increase in the erythrocyte sedimentation rate. Electrocardiograms taken at frequent intervals showed slight and inconclusive changes. These were practically confined to the T-wave in Lead III. The S-T interval returned to the base line the day after the attack.

On May 17, 1939, six weeks after her attack, she was given three units of Iletin after breakfast. Her fasting blood sugar was 132 mg. per cent. Twenty-five minutes later she went into a profound shock. Fifteen minutes after the beginning of this attack the blood sugar was 143 mg. per cent.

Twenty minutes after the beginning of the attack the pulse was barely perceptible and very rapid. The blood pressure was 65 systolic and 0 diastolic. The patient was cyanotic, dyspneic, and exhibited irregular sighing respirations. Her skin was cold, moist, and clammy. She showed tremors and convulsive movements. Adrenalin injected subcutaneously gave her slight relief; she was drowsy for several hours. The blood pressure six hours later was 85 systolic and 65 diastolic. The white blood cell count eight hours later was 9,200. The electrocardiogram shortly after the onset of the attack showed minimal changes. An allergic work-up was done one week later. Table I contains the skin tests.

The patient gave positive reactions to all tested brands of insulin, including crystalline insulin. She did not react to pork, beef, and lamb extracts. These tests show clearly that she was allergic to pure crystalline insulin. Passive transfer was attempted. The result was inconclusive as the normal test person gave positive intradermal reactions also in untreated skin areas.

TABLE I

TEST SUBSTANCE	METHOD OF TESTING		
	SCRATCH	PUNCTURE	INTRADERMAL
Lilly's Iletin 40 U.	+++	+++	
Lilly's Iletin special (Beef) 20 U.	+++	+++	
Squibb's insulin 20 U.	±	+ (+)	
Stearn's insulin	±	+	
Lilly's Iletin zinc 40 U.	++	+ (+)	
Stearn's zinc insulin	++	++	
Lilly's protamine zinc insulin 40 U.	++ (+)	++	0
Pork	0	0	0
Beef	0	0	0
Lamb	0	0	0

SUMMARY

The injection of 20 units, and later of 3 units, of Iletin (Lilly) in a 65-year-old arteriosclerotic hypertensive diabetic patient gave rise to a syndrome strongly simulating coronary occlusion. Reviewing the case from the viewpoint of a possible allergy, it is clear that we are dealing with the symptoms of anaphylactic shock. Skin tests corroborated this assumption.

REFERENCE

1. Herzstein, Joseph, and Pollack, Herbert: *J. Mt. Sinai Hosp.* 6: 3, 1939.

THE ROLE OF METALS IN CARBOHYDRATE METABOLISM*

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EXTENSIVE experimental and clinical investigations concerning the effect of various metals-insulin mixtures have been recorded by many workers. Thus, it is well known that the addition of 0.1 per cent of zinc to insulin delays and prolongs the action of the latter, while the addition of 0.9 per cent of zinc completely inhibits the insulin effect. This phenomenon brings up the question of the possible basic role of the metals in carbohydrate metabolism under both physiologic and pathologic conditions. To evaluate the state of our present knowledge the important contributions in this field are summarized.

Sodium.—Magenta¹ studied the effect of sodium fluoride on the glyceemic levels of dogs. He found that the intravenous injection of sodium fluoride did not provoke a glycosuria or a hyperglycemia in them. His observations supplement those previously described by Goldenberg,² who observed a glycosuria among both lambs and horses subjected to prolonged ingestion of sodium fluoride.

In 1925 Abelin and Goldener³ investigated the effect of certain sodium salts on the hypoglycemic action of insulin in animals. The oral administration of 2 Gm. of monosodium phosphate ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$) or 2 Gm. of a mixture of sodium chloride, sodium bicarbonate, and sodium sulfate produced a marked augmentation of the hypoglycemic effect of 4 to 6 units of insulin given simultaneously. The use of 2 Gm. of disodium phosphate ($\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$) has no appreciable effect on insulin action (Abelin and Goldener³).

Apparently these authors did not understand nor question the reason why disodium phosphate did not equal or increase the hypoglycemic effect of insulin action. It is clear that they did not take into consideration the twelve times larger amount of water crystallization in the disodium phosphate molecule, thus producing a unit containing much less sodium per gram than monosodium phosphate. It is quite probable that if this fact were taken into account, the effect of the disodium salt would be equal to or greater than that of the monosodium phosphate. The fact that the effect of the sodium salt mixture was equal to that of the monosodium phosphate appears to indicate that the active portion of the molecule is the sodium ion. Abelin and Goldener³ did not determine whether the hypoglycemic effect of the sodium salts is a direct effect on carbohydrate metabolism or merely an indirect result of their effect on insulin.

This problem has been further investigated by McQuarrie, Thompson, and Anderson,⁴ who demonstrated that the hypoglycemic effect of sodium salts is a direct one on carbohydrate metabolism, since a drop in blood sugar level of persons with diabetes could be produced without the simultaneous injection of

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insulin. They also studied the effect of excessive ingestion of sodium salts on the carbohydrate metabolism of four diabetic children, between 13 and 15 years of age. In each case it was found that the ingestion of approximately 60 Gm. of sodium chloride daily produced a marked decrease in the amount of glucose appearing in the urine. In the case of a 15-year-old boy who required 32 units of insulin daily, the administration of 60 Gm. of salt decreased the urinary glucose from 73 Gm. to a minimum of 13 Gm. after two days. After seven days the salt was discontinued and the urinary glucose returned to the original level within three days. Although the fasting blood sugar level was decreased and the respiratory quotient was increased by salt administration, the appearance of the sugar tolerance curve did not show any change. Less insulin was required during the period of salt administration, since typical insulin reactions occurred when the insulin dosage was not decreased. It was shown that the effect was due to the sodium ion, since the use of other sodium salts produced similar effects while other chlorides did not. The administration of excessive amounts of sodium chloride did not produce hypoglycemia in a normal control subject. The mechanism of the sodium action is not known. It does not appear that the phenomenon can be satisfactorily explained solely on the basis of water and glucose retention in the tissues. McQuarrie and associates⁴ investigated the possible importance of cortical extract in explanation of the sodium effect; e.g., after producing a marked decrease in the glycosuria of a person with severe diabetes, these workers substituted adrenal cortical extract for the sodium chloride and found that the degree of glycosuria returned toward the presaline level.

Potassium.—The action of the potassium ion on carbohydrate metabolism is, in general, directly opposed to that of the sodium ion. Potassium chloride, when administered alone, tends to decrease carbohydrate tolerance and to raise the blood sugar level, both in man and experimental animals. As in the case of sodium, the exact mechanism involved is not definitely known. Rathery, Bertoliatti,⁵ and others found that the blood potassium level of diabetic individuals is characteristically elevated. McQuarrie⁴ noted a drop in serum potassium of diabetic persons while on a high sodium chloride intake. The high vegetable diet of the average diabetic person is high in potassium content. McQuarrie⁴ and his co-workers have reported an increase averaging 50 per cent in the excretion of glucose in persons with diabetes while being fed extra amounts of potassium chloride. While receiving 10 to 20 Gm. of potassium chloride, a low sodium diet, and the carbohydrate intake kept constant, the urine glucose excretion increased from 60 to 70 Gm. to 70 to 100 Gm. daily. They found that the oral administration of potassium chloride to a diabetic patient receiving large amounts of sodium chloride resulted in an elevation of the glucose excretion. The results appeared to indicate that the administration of one milliequivalent of potassium neutralized the effect of three milliequivalents of sodium.

The antagonistic effects of sodium and potassium were similar in diabetic persons receiving insulin and those not receiving insulin therapy. McQuarrie and co-workers⁴ report the relief of symptoms due to hypoglycemia in an acromegalic person by the administration of potassium chloride.

Calcium-Magnesium.—Kiyohara, Morita, and Muta⁶ investigated the effect of potassium on the glycemic levels of dogs by means of perfusion of the isolated pancreas with blood to which potassium chloride had been added. The addition

of potassium chloride (0.1 Gm. per 100 c.c. of blood) modified the content of the blood sugar. Kiyohara, Morita, and Muta⁸ stated that the potassium ion did not exercise any direct influence on the insular tissue of the pancreas. Their work clearly showed that in rabbits an injection of 3 c.c. of potassium chloride solution (2 Gm. per 100 c.c. per kilogram of body weight) established a hyperglycemia of such severity that it lasted for ninety minutes. This observation corresponds to the findings of Hochfeld⁷ and others. The injection of 3 c.c. of a solution of calcium chloride (5 parts per 100 c.c. per kilogram of body weight) also provoked a hyperglycemia, but the latter was less accentuated than that caused by potassium chloride. Barath⁸ and Schenk⁹ report a hypoglycemia in the same type of experiment. Previous injection of atropine did not modify the hyperglycemia provoked by the injection of either potassium or calcium. Kiyohara⁸ found that the hyperglycemia could not be induced by either potassium or calcium ions after the extirpation of both adrenals as it could before the extirpation. Kiyohara⁸ attributed the hyperglycemia brought about by these ions to the action on the adrenals themselves. Hazard¹⁰ studied the action of calcium and magnesium on potassium-provoked hypertension and hyperglycemia. He demonstrated that large doses of potassium chloride cause a discharge of adrenalin, which in turn produces a hypertension and a hyperglycemia. He showed that magnesium prevents potassium from exercising its adrenalin action while calcium allows it to provoke the adrenalin discharge but diminishes its hyperglycemic effect. The reduction of adrenalin glycosuria by calcium has been noted by various authors, some of whom even advocate the use of calcium salts in the treatment of diabetes in man. Among these, Asada¹¹ has shown that, in rabbits, repeated intravenous injections of small amounts of calcium chloride reduced the hyperglycemia due to adrenalin by 13 per cent. He also noted a 28 per cent smaller increase of blood sugar in rabbits which had been injected intravenously with 5 c.c. of 2 per cent potassium chloride solution, together with 3 mg. of adrenalin per kilogram of body weight subcutaneously, than with the same amount of potassium chloride. This question merits more study because the potassium in blood has been found to be higher in certain forms of diabetes. Further as to the effect of calcium on blood sugar, Cantarow, Brundage, and Housel¹² found that the intravenous injection of calcium gluconate and calcium levulinate in doses of 3.7 to 9.9 mg. of calcium ion per kilogram of body weight was followed by a decrease of 10 to 31 mg. per 100 c.c. in blood sugar within five to fifteen minutes. No inhibitory effect on insulin action was observed in the animal experiments of Fazekas and Himwich¹³ when 10 mg. of calcium chloride or 10 mg. of magnesium sulfate were added to insulin prior to injection. It is interesting to note that although calcium, magnesium, and zinc belong in the same group in the Mendeléeff table, only zinc possesses the inhibitory effect on insulin action.

Nickel-Cobalt.—Bertrand and Macheboeuf¹⁴ found the pancreas rich in nickel and cobalt. Labbe, Roubeau, and Nepveux¹⁵ confirmed the results obtained by Bertrand and Macheboeuf¹⁴ in their experiments on rabbits in which they showed that the addition to insulin of a trace of a nickel salt or of a cobalt salt intensifies and prolongs the hypoglycemic effect. Labbe, Roubeau, and Nep-

veux¹⁵ then sought to determine the action of nickel and cobalt salts on the hypoglycemic power of insulin on diabetic individuals. They conducted two series of experiments. In the first series isolated injection of nickel chloride and cobalt chloride salts in isotonic solution into diabetic individuals with malnutrition and acidosis showed that the blood sugar level is not modified by cobalt and nickel salts. Identical injections made in normal subjects showed no modification in their glycemic levels.

In the second series of experiments they sought to find whether the injection of metallic solutions would influence the hypoglycemic power of insulin on diabetic individuals in the same manner as on animals. They administered half units of insulin per kilogram of body weight to eight diabetic individuals with acidosis and malnutrition, and to one diabetic patient without malnutrition. They varied the doses of nickel and cobalt chloride injected per kilogram of body weight, the first series of patients receiving 0.1 mg. of nickel chloride; the second series, 1 mg. of nickel chloride; the third series, 1 mg. of cobalt chloride. For each patient it was possible to make two series of observations, the first with insulin alone, the second with the same quantity of insulin added to the metallic solution. For each, the two curves of glycemia with and without nickel or cobalt salts were such that no reinforcement of the action of insulin, either in intensity or duration, could be noted. They found that the injection of cobalt or nickel salts without insulin, whether in healthy or in diabetic individuals, was without hypoglycemic effect as Bertrand and Macheboeuf¹⁴ had shown to be the case with animals. They reported that action of insulin in association with nickel or cobalt salts on diabetic persons varied from that observed in healthy rabbits and dogs, a difference perhaps due to a dissimilar metabolism of the glucides in healthy animals and diabetic human beings. Magenta¹ in 1926 obtained in dogs results similar to those obtained by Blatherwick and Sahyun¹⁶ in rabbits (1927). The latter reported a slight increase of glycemia when metallic salts alone were used. Metallic salts used simultaneously with insulin exercised little if any preventive action on the hypoglycemia of dogs, and never increased the hypoglycemic effect of insulin.

Zinc.—Hove, Elvehjem, and Hart¹⁷ investigated the relationship of zinc to certain aspects of carbohydrate and protein metabolism in rats. Glucose tolerance tests were conducted by means of pipette-feeding of 0.2 Gm. of glucose per 100 Gm. of body weight after a five-hour starvation period. Tolerance curves obtained from zinc-deficient rats differed from rats on a normal diet. Liver glycogen determinations on both zinc-deficient rats and control rats revealed no difference in respect to liver glycogen. In the case of the zinc-deficient rats, these investigators found a distinct delay in the time interval necessary for the blood nitrogen to reach its maximum value. The distinct irregularity of the glucose tolerance curves obtained from zinc-deficient rats is explained as being due to a delay in the absorption rate of carbohydrate and protein from the gastrointestinal tract.

In dogs fed zinc oxide, D'Amore, Falcone, and Maramaldi¹⁸ observed occurrences of severe glycosuria. Salant and Wise¹⁹ also report glycosuria following the administration of zinc acetate and zinc malate, both orally and intravenously.

These observers explain this phenomenon on the basis of an increased permeability of the kidneys to sugar (renal diabetes).

Drinker, Thompson, and Marsh²⁰ state that glycosuria was not present in cats which had received zinc oxide in the form of a dry powder mixed with food. However, they did encounter glycosuria in two of three dogs subjected to the same method of zinc oxide administration. Curiously, gross and microscopic examination following autopsy revealed fibrous change in the pancreas of the zinc-treated cats and none in the pancreases of the zinc-treated dogs. No other organs of these animals showed pathologic change. The authors state that the amount of zinc oxide administered is greatly in excess of that usually encountered by man. Impressed by the fibrous changes in the pancreas of zinc-treated cats, and knowing through their own researches the close chemical relationship between insulin and zinc, Scott and Fisher²¹ estimated the insulin content and the zinc content of the pancreases of cats maintained on a basal diet, and also a basal diet to which zinc oxide had been added. They, too, found that the pancreas was greatly involved in the zinc-treated cats through degenerative changes in the acinar tissues. Nodular pancreatic glands with bands of fibrous tissue replacing areas of acinar tissue were observed. The islet tissue of the pancreas in both the zinc and the control groups showed no change. Amyloid deposits in the spleen and calcification in the cortex of the adrenals were noted in many of the zinc-treated cats. The liver and kidneys in both groups showed that a large amount of fat was present.

Since these changes occurred in both groups, it is not certain that zinc feeding was their specific cause. No changes were found in other tissues examined. They assert that heavy zinc feeding did not alter the total insulin content of the pancreas. Their results appear to indicate an increase in the amount of insulin per gram of pancreas, while the total insulin content remained unchanged. It was observed that on a basal diet the cat pancreas contains more zinc per gram of tissue than the liver.

The livers of zinc-fed cats contained nearly twice as much zinc per gram of tissue as the pancreases, although the cats on a basal diet contained a higher percentage of zinc per gram of tissue in the pancreas than in the liver. On the zinc diet insulin assays showed that 2.56 units of insulin were present per gram of pancreas, while on the basal diet only 1.68 units of insulin per gram of pancreas were present. The total insulin content of both groups was nearly the same (10.40 units on the zinc diet and 11.52 units on the basal diet).

Scott and Fisher²² assayed the pancreases of nondiabetic human individuals who came to autopsy and found that 1.7 units of insulin per gram of pancreas were present. In diabetic individuals who came to autopsy, they noted that the pancreas contains less than 0.4 unit of insulin per gram of pancreatic tissue. It was observed that the average zinc content of the diabetic pancreas is one-half that of the normal pancreas. The zinc per gram of pancreas in the diabetic person assays at 0.07; in the nondiabetic person, the value is 0.14. No marked differences in zinc concentration in the livers of diabetic and nondiabetic individuals were observed. It is of interest to note the marked reduction of both insulin and zinc in the pancreas of the diabetic individual. Scott and Fisher²²

suggest from this observation the possibility that a part of the zinc in the pancreas might be concerned with the storage and utilization of insulin.

Zinc Insulin Mixtures.—Zinc has been found to be present in the pancreas; it has also been found in all commercial preparations of insulin examined by Scott and Fisher.²³ Insulin solutions to which 0.02 per cent of zinc chloride are added, or equivalent amounts of zinc in the form of zinc acetate or zinc sulfate, demonstrated only 40 per cent of the original hypoglycemic activity when injected into mice. It was noted that convulsions occurred later in the group of mice that were given zinc insulin solutions than in the control group given insulin alone. This observation suggested that the action of insulin was delayed by the presence of the zinc salt. In rabbits, when the zinc insulin mixture was so adjusted that 0.24 per cent of zinc chloride was present, the action of insulin was also greatly delayed. These authors suggested that the zinc added to insulin altered the solution, so that a greatly delayed hypoglycemic action was produced. Rabbits receiving insulin alone yielded the lowest blood sugars two hours after the injection of insulin, and blood sugars reached the normal level in ten hours. Rabbits given zinc insulin showed a gradual fall in the blood sugar values during the first five hours, and blood sugar values were still below the normal level at the end of a ten-hour period. Injections of zinc sulfate solutions alone did not alter blood sugar response during an observation period of seven hours. It appears that the delayed hypoglycemic action of zinc-insulin mixtures is dependent upon some process which takes place in the solution before it is injected.

Fazekas and Himwich¹³ observed no alteration of the typical action in either dogs or cats when insulin and zinc sulfate are injected independently into separate subcutaneous areas. Injections of zinc sulfate, the same concentrations as above but without insulin, had no effect whatsoever on blood sugar levels. When 0.9 per cent zinc was used in a zinc-insulin mixture, complete inhibition of insulin action resulted.

Copper.—Ussolzew²⁴ found that intravenous injections of 0.5 mg. and 1 mg. of copper nitrate caused a lowering of blood sugar in rabbits starved for twenty-four hours previous to injection. Tkachenko²⁵ found in rabbits that the administration of 0.1 mg. of copper per kilogram of body weight caused a hypoglycemia in rabbits.

Schnetz²⁶ observed the effect of copper on carbohydrate metabolism both through studies of the isolated liver and on the intact animal. His results show that copper causes a definite lowering of the ability of adrenalin to cause mobilization of glycogen from the liver, but does not disturb the normal glycolytic mechanism. He also found that the oral administration of 20 mg. of copper sulfate per day for a period of days in normal individuals reduced both the hyperglycemia caused by the injection of 0.5 mg. of adrenalin and the hyperglycemia produced by the administration of 30 Gm. of dextrose orally. In his experiments, equivalent amount of iron salts did not influence a hyperglycemia identically induced.

Copper salts were given over a period of weeks to four persons suffering from severe diabetes mellitus. A noticeable reduction in the amount of insulin necessary to control their diabetes was observed, as was also a lowering of blood

sugar and urinary sugar and a disappearance of acetone bodies. This action of copper in reducing blood-sugar is marked in cases of high blood sugar values. When blood sugar values are within normal limits, little effect is observed. These experiments (copper-adrenalin-dextrose) on normal individuals, and those made in the course of diabetes mellitus, appear to indicate that it is to hepatic action that copper-reduction of blood sugar values is due. This hypothesis is made on the basis of the following theory: copper administered by mouth is thought to be transported by the reticulo-endothelial system to the liver. Muller²⁷ believes that copper so alters the glycogenolytic mechanism of the liver that it does not respond in its usual way to adrenalin mobilization of glycogen action, with the resulting rise in blood sugar levels. Muller²⁷ believes that copper makes for stabilization of hepatic glycogen function. Schnetz²⁶ states as a hypothesis that copper is a catalyst which helps the oxidation of carbohydrates through hepatic or extrahepatic action.

Since at present we do not know where copper is distributed in the liver nor how long it remains in it, nor what chemical unions and reactions it engages in, we can only say that it appears that the real mechanism is not yet explained. The foregoing interesting theories must remain unproved hypotheses until more data have been made available.

Aluminum Insulin Mixtures.—When 11.2 mg. of aluminum chloride were mixed with ten units of insulin before injection, Fazekas and Himwich¹³ observed no decrease in blood sugar values in the animals. When 5.6 mg. of aluminum chloride were mixed with ten units of insulin, a decrease in blood sugar values occurred, showing that the aluminum chloride at this concentration does not abolish insulin activity. Since Scott and Fisher²³ showed that potassium alum had no inhibitory effect on insulin, aluminum chloride apparently differs from potassium alum in its inhibitory effect on insulin.

Ferric Chloride Insulin Mixtures.—Maxwell and Biscoff²⁸ investigated the action of basic ferric salts in prolonging insulin hypoglycemia. Insulin was added to a dilute aqueous solution of ferric chloride which had been neutralized to the point of precipitation. In both rabbits and rats the addition of basic ferric chloride to insulin augmented the insulin action. The blood sugar of the control animals receiving insulin alone approached the normal at the fifth hour, while the blood sugar of the group that received insulin plus ferric chloride approached the normal value at the eighth hour.

Manganese.—Pignatari²⁹ noted the effect of manganese sulfate on the carbohydrate metabolism of rabbits. When 0.50 Gm. of manganese sulfate in physiologic solution per kilogram of body weight was injected daily, the animals died after the fourth or fifth injection. With doses of 0.10 Gm. of manganese sulfate, the animals remained alive for approximately one and one-half months. The first symptom of the manganese intoxication appeared after 0.80 Gm. of manganese sulfate had been administered. Pignatari²⁹ determined the fasting blood sugar level in each rabbit before the poisoning and on alternate days during the course of the intoxication. The method used for the determination of the blood sugar values was that of Bang-Condorelli.

The blood sugar values during the intoxication with manganese sulfate showed a decrease in the fasting blood sugar levels. In the final period of the

intoxication the glycemic level was so markedly decreased that in some cases the fasting blood sugar level was approximately one-half of the normal.

Pignatari²⁹ carried out glucose tolerance tests on these animals during the various stages of the intoxication with manganese sulfate. The blood sugar levels were determined until the fourth hour after the administration of the glucose. The principal characteristic of the glycemic reaction in rabbits intoxicated with manganese was the prolongation of the hyperglycemia. The hyperglycemia was due to the diminished capacity of the liver to absorb the circulating glucose and to synthesize glycogen. The hypoglycemia observed in the course of the intoxication with manganese can best be explained by a deficient and impaired glycolytic mechanism of the liver. Both the delayed hepatic glycogenesis and the deficient and impaired glycogenolysis can best be explained by changes produced by manganese sulfate on the liver.

Lead.—Von Ludwig Schmidt-Kehl³⁰ reports that rabbits fed a daily dose of 10 mg. of lead oxide per kilogram of body weight over a period of two or four months developed chronic lead poisoning. Five normal rabbits were used as controls. The administration of 2 per cent of the body weight of levulose in normal control animals, after a day of starvation, showed a rise of 16 per cent in the blood sugar values. In the five animals with chronic lead poisoning, the increase in blood sugar values rose to 35 per cent. These determinations were made according to the method of Hagedorn and Jensen. Histologic examinations revealed no pathologic changes present in either normal or leaded animals. It was concluded that in chronic lead poisoning of rabbits, even without the presence of any anatomic and histologic changes in the liver, there is already present undiscernible damage, as evidenced by increased blood sugar values in the leaded animals.

Cavagliano³¹ observed the occurrence of glycosuria in patients during episodes of lead colic. He reports that the administration of 1.5 Gm. of glucose per kilogram of body weight to patients recovering from lead colic resulted in a glycosuria that manifested itself for a period of four hours. After considering alimentary glycosuria and renal glycosuria as possible explanations for this mechanism, he concludes, "The glycosuria indicates the functional disturbance of the liver caused by lead."

It has been demonstrated that injections of lead, whether in solution or in a colloidal state, localize chiefly in those organs that are rich in reticulo-endothelial elements, and hence particularly in the liver. Colloidal lead, like mineral colloids in general, when injected, are prevalently deposited in the liver. The variations of the glycemic levels can be studied before and after the injection of these colloids.

Merlini³² injected carefully standardized colloidal lead preparations into the marginal veins of rabbits. The normal glycemic levels of rabbits were determined in the control group. The blood sugar values varied from 116 mg. to a minimum of 81 mg. of glucose per 100 c.c. of blood. The average value of the blood sugar determinations in this control group was 83.9 mg. of glucose per 100 c.c. of blood. Merlini noted that rabbits which had received 0.021 Gm. of lead per kilogram of body weight after a few hours had blood sugar values

ranging from 54 to 58 mg. of glucose per 100 c.c. of blood. When the dose of the colloidal lead solution was reduced so that 0.01094 Gm. of lead per kilogram of body weight was injected daily for a period of five days, and then fasting blood sugar levels were determined, a hypoglycemia was noted. The level of this hypoglycemia was never found to be much below 50 mg. of glucose per 100 c.c. of blood. Merlini's data show that the rabbits reacted to colloidal lead with hypoglycemic reaction according to the dose administered.

Phosphotungstic Acid—Phosphomolybdic Acid.—Mukherjee³³ in 1925 noted the effects of the phosphotungstates and phosphomolybdates on carbohydrate metabolism when taken internally. Experimentally, phospho-24-tungstic acid, phosphomolybdic acid, molybdic acid, silicotungstic acid, sodium tungstate, and ammonium phospho-18-tungstate when taken by mouth lower the glycemie level in both rabbits and diabetic human beings. The experiment using diabetic patients was as follows: Three hours after administration of insulin there was an average blood sugar drop of 125.3 mg. per 100 c.c. of blood. Three hours after intake of 0.9 Gm. of phosphotungstic acid, an average blood sugar drop of 90 mg. per 100 c.c. of blood resulted. A diuretic action of these substances was noted while demonstrating the above hypoglycemic effects. An oxidizing action is observed when 0.1 Gm. of phosphomolybdic crystals in a 5 c.c. solution of benzidine in acetic acid turns blue, and becomes deeper in color when hydrogen peroxide is added. Benzidine and phosphomolybdic acid alone give a positive reaction. The allied substances give a positive reaction when added to alkaline phenolphthalein reagent. These substances, Mukherjee believes, act like oxidizing enzymes; and the oxidizing action associated with the hypoglycemic reaction suggests that oxidation of sugar in the tissues may produce hypoglycemia.

Mercury.—Richter³⁴ observed the presence of glycosuria associated with hyperglycemia in animals after the administration of sublimate of mercury. Frank³⁵ found that the intravenous injection of 3 mg. of bichloride of mercury into rabbits was followed by a marked glycosuria without change in the glycemie level.

Hunter and Roberts,³⁶ on the other hand, claim that in addition to the widespread tubular necrosis, distinct changes interpreted as evidences of injury can be demonstrated in the glomerular basement membranes of kidneys damaged by mercuric chloride. These alterations in the basement membrane may perhaps play an important role in the renal disturbances induced by the salts of mercury. In man, in the course of corrosive sublimate intoxication, Lepine³⁷ has observed glycosuria.

Uranium.—Lepine and Boulud,³⁸ Fleckseder,³⁹ Pollak,⁴⁰ Frank,³⁵ and Luzatto,⁴¹ reported a glycosuria variable in degree produced by uranium salts. In fasting animals, it was less and appeared later than in well-fed ones. In dogs, Frank³⁵ found that glycosuria prevailed from eight to eleven days after a single injection. Whitney⁴² states that in the acute nephritis produced by uranium there is damage to the tubular epithelium of the kidneys and to the capillaries. There is generalized capillary damage resulting in decreased permeability of the walls. Reports as to sugar concentration in the blood varied from hyperglycemic to hypoglycemic levels.

Chromium.—Vernon,⁴³ first, and Kossa⁴⁴ later called attention to the production of glycosuria by chromium salts. Kossa's studies determined the amount of sugar in both the blood and the urine of animals subjected to his experiments. He found no hyperglycemia in animals poisoned with chromate. Other investigators report contradictory findings, both higher and lower than those of Kossa. Luzzatto⁴¹ administered potassium chromate to well-fed and fasting animals and observed glycosuria and albuminuria following administration.

In addition to widespread tubular necrosis, distinct changes, interpreted as evidences of injury, can be demonstrated in the glomerular basement membranes of kidneys damaged by chromium.³⁶

Tellurium.—Luzzatto⁴¹ states that tellurium salts, in particular sodium tellurate, may cause glycosuria and albuminuria in animals, sometimes reaching 1 per cent sugar concentration. Albuminuria sets in first, followed within forty-eight hours after injection by a glycosuria which may last five days. Observations made on both fasting and well-fed animals indicated no increase in glycemie levels. Kaiwa⁴⁵ demonstrated that b-dimethyltelluronium dichloride, administered subcutaneously as well as intravenously, was effective in provoking hyperglycemia. The hyperglycemia first begins to appear one-half to one hour after injection, and lasts from two to four hours. Additional experiments were made on rabbits of which the splanchnic nerves had been bilaterally sectioned in order to observe whether the hyperglycemia due to b-dimethyltelluronium dichloride is of central origin. Results were identical, showing no involvement of the central mechanism. However, experiments on bilaterally suprarenalectomized rabbits showed a lower level of hyperglycemia than that found in either the normal or the splanchnicotomized animals.

COMMENTS

It is apparent that with the exception of sodium, potassium, nickel, and cobalt, the investigations of the direct effect of metals on carbohydrate metabolism have consisted, for the most part, of animal experimentation. Routine glucose tolerance tests in clinical cases of metal intoxications will probably yield interesting and significant information in this field. The data thus obtained will aid greatly in elucidating the physiologic and pathologic relationships of the metals and carbohydrate metabolism.

SUMMARY

1. The sodium ion depresses the blood sugar level in rabbits. In juvenile diabetic patients the administration of sodium salts decreases both blood sugar level and glycosuria. The mechanism of this action is unknown.
2. Potassium salts increased the blood sugar concentration and glycosuria of juvenile diabetic persons. This effect appears to be due to a stimulating effect on adrenalin discharge and is inhibited by magnesium.
3. There is no unanimity of opinion concerning the effect of calcium on the glycemie levels of various experimental animals. Calcium salts appear to depress the hyperglycemic effect of epinephrine in animals.

4. Nickel and cobalt salts, when added to insulin, delay the effect of the latter in normal rabbits and dogs, but not in diabetic human beings. Nickel and cobalt salts, when given alone, have no effect on blood sugar level of man or animals.

5. Zinc-deficient diet delays the absorption of carbohydrate from the gastrointestinal tract of rats. Zinc oxide has been found to produce glycosuria in dogs and pancreatic fibrosis in cats. The zinc content of the pancreases of diabetic persons is greatly below normal.

6. Zinc, aluminum, ferric chloride, nickel, or cobalt, when mixed with insulin, delays the action of the latter; calcium, magnesium, and potassium alum do not delay the effect of insulin when mixed with it.

7. Lead and manganese decrease the fasting blood sugar levels.

8. Phospho-24-tungstic acid, phosphomolybdic acid, molybdic acid, silico-tungstic acid, sodium tungstate, and ammonium phospho-18-tungstate appear to cause a decrease in blood sugar of diabetic persons.

9. Glycosuria has been observed after the administration of mercury, uranium, and chromium.

10. The administration of copper salts, both in man and animals, causes a decrease in the ability of adrenalin to mobilize liver glycogen.

REFERENCES

1. Mageata, M. A.: Action du fluorure de sodium sur la glycémie, *Compt. rend. Soc. de biol.* 98: 169, 1928.
2. Goldenberg: 3^e Congrès national de médecine, t. vi, fasc. 2, 663 Juillet, 1926.
3. Abelia, J., and Goldener, E.: Influence of Electrolytes on Action of Insulin, *Klin. Wchnschr.* 4: 2446, 1925.
4. McQuarrie, L., Thompson, W. H., and Anderson, J. A.: Effects of Excessive Ingestion of Sodium and Potassium Salts on Carbohydrate Metabolism and Blood Pressure in Diabetic Children, *J. Nutrition* 11: 77, 1936.
5. Rathery, F., and Bertoliatti, J.: Le potassium du sang dans les différentes formes du diabète sucré, *Compt. rend. Soc. de biol.* 117: 875, 1934.
6. Kiyohara, K., Morita, M., and Muta, S.: De l'influence des ions K et Ca sur la teneur en sucre du sang, *Compt. rend. Soc. de biol.* 120: 1011, 1935.
7. Hochfeld, O.: Ueber den Einfluss von Kalk- und Kalisalsen auf den Blutzucker, *Ztschr. f. d. ges. exper. Med.* 37: 119, 1923.
8. Barath, E.: Calciumwirkung und Blutzucker, *Klin. Wchnschr.* 3: 1618, 1924.
9. Schenk, P.: Ueber den Einfluss der intravenösen Infusion hypertensischer Natrium- und Calciumchloridlösungen auf den Blutzuckerspiegel, die Diurese und die Durchlässigkeit der Nieren, *Ztschr. f. d. ges. exper. Med.* 12: 269, 1921.
10. Hazard, René: Action de l'ion calcium sur l'hypertension et sur l'hyperglycémie provoquée par l'ion potassium, *Compt. rend. Soc. de biol.* 121: 1036, 1936.
11. Asada, T.: Einfluss des Kaliumchlorids auf die Adrenalinhyperglykämie und -lactacidämie, *Mitt. a. d. med. Akad. zu Kioto* 15: 1140, 1935.
12. Cantarow, A., Brundage, J. T., and Honsel, E. L.: Blood Sugar, Inorganic Phosphorus and Phosphatase Activity Following the Intravenous Injection of Calcium Salts, *Am. J. Physiol.* 115: 1, 1936.
13. Fazekas, J. F., and Himwich, H. E.: Effect of Zinc and Aluminum on Hypoglycemic Action of Insulin, *J. Pharmacol. & Exper. Therap.* 58: 260, 1936.
14. Bertrand, G., and Macheboeuf, M.: (1) Influence du nickel et du cobalt sur l'action exercée par l'insuline chez le chien, *Compt. rend. Acad. d. sc.* 183: 5, 1926; (2) Nickel, cobalt et diabète, *Compt. rend. Acad. d. sc.* 183: 257, 1926; (3) Sur la teneur relativement élevée du pancréas en nickel et en cobalt, *Compt. rend. Acad. d. sc.* 182: 1305, 1926; (4) Influence du nickel et du cobalt sur l'action exercée par l'insuline, chez le lapin, *Compt. rend. Acad. d. sc.* 182: 1504, 1926.
15. Labbe, M., Roubeau, H., and Nepveux, F.: Action de sels de nickel et de sur le pouvoir hypoglycémiant de l'insuline chez le diabétique, *Compt. rend. Acad. d. sc.* 186: 181, 1928.
16. Blatherwick, N. R., and Sahyun, M.: Effect of Cobalt on Insulin Hypoglycemia in Rabbits, *Am. J. Physiol.* 81: 560, 1927.
17. Hove, E., Elvehjem, C. A., and Hart, E. B.: Physiology of Zinc in Nutrition of Rat, *Am. J. Physiol.* 119: 768, 1937.

18. D'Amore, L., Falcone, C., and Marmaldi, L.: Action toxique et alterations anatomiques produites par l'ingestion de l'oxyde de zinc, *Compt. rend. Soc. de. biol.* 44: vol. 4: s. 9, 335, 1892.
19. Salant, W., and Wise, L. E.: The Production of Glycosuria by Zinc Salts, *J. Biol. Chem.* 34: 447, 1918.
20. Drinker, K. R., Thompson, P. K., and Marsh, M.: Investigation of Effect of Long-Continued Ingestion of Zinc, in Form of Zinc Oxide, by Cats and Dogs, Together With Observations Upon Excretion and Storage of Zinc, *Am. J. Physiol.* 80: 31, 1927.
21. Scott, D. A., and Fisher, A. M.: Studies on Pancreas and Liver of Normal and Zinc-Fed Cats, *Am. J. Physiol.* 121: 253, 1938.
22. Scott, D. A., and Fisher, A. M.: Insulin and Zinc Content of Normal and Diabetic Pancreas, *J. Clin. Investigation* 17: 725, 1938.
23. Scott, D. A., and Fisher, A. M.: Effect of Zinc Salts on Action of Insulin, *J. Pharmacol. & Exper. Therap.* 55: 206, 1935.
24. Ussolzew, S.: Ueber den Einfluss von Kupferdarreichung auf den Blutzuckerspiegel, *Biochem. Ztschr.* 276: 431, 1935.
25. Tkaehenko, L.: Ueber den Einfluss von Metallsalze auf den Blutzuckergehalt, *J. Orient. Med.* 7: 66, 1927.
26. Schnetz, H.: Ueber den Einfluss des Kupfers auf den Kohlehydratstoffwechsel des Menschen, *Ztschr. f. klin. Med.* 129: 739, 1936.
27. Muller, A. H.: Ausführliche Literaturangaben über Kupfer, *Med. Welt.* 2: 299, 1935; *Ergebn. d. inn. Med. u. Kinderh.* 48: 444, 1935.
28. Maxwell, L. C., and Bischoff, F.: Augmentation of Physiologic Response to Insulin, *Am. J. Physiol.* 112: 172, 1935.
29. Pignatari, F.: Glicemia e lipemia nella intossicazione de Manganese, *Folia med.* 18: 484, 1932.
30. Selmidt-Kehl, L.: Ueber die Schädigung des Kohlehydratstoffwechsels der Leber bei chronischer Bleivergiftung, *München. med. Wchnschr.* 80: 2003, 1933.
31. Cavagliano, B.: Glicosurie e diabete in rapporto alla medicina assicurativa, *Med. del lavoro* 20: 407, 1929.
32. Merlini, E.: Ricerche sperimentali sulla glicemia nell'avvelenamento da piombo colloidale, *Arch. Ist. biochim. ital.* 5: 395, 1933.
33. Mukherjee, H. N.: Hypoglycemic and Other Actions of Phosphotungstic Acid. Phosphomolybdic Acid and Allied Substances, *Biochem. J.* 30: 1583, 1936.
34. Richter, P. F.: Zur Frage des "Nierendiabetes," *Deutsche. med. Wchnschr.* 25: 840, 1899.
35. Frank, E.: Ueber experimentelle und klinische Glykosurien renalen Ursprungs, *Arch. f. exper. Path. u. Pharmacol.* 72: 387, 1913.
36. Hunter, W. C., and Roberts, J. M.: Glomerular Changes in Kidneys of Rabbits and Monkeys Induced by Uranium Nitrate, Mercuric Chloride, and Potassium Bichromate, *Am. J. Path.* 8: 665, 1932.
37. Lepine, R.: Les glycosuries toxiques, *Arch. de méd. expér. et d'anat. path.* 15: 129, 1903.
38. Lepine, R., and Boulud: Sur L'absence d'hyperglycémie dans la Glycosurie uranique, *Rev. med.* 24: 1, 1904.
39. Fleckseder, R.: Ueber Hydrops und Glykosurie bei Uranvergiftung, *Arch. f. exper. Path. u. Pharmacol.* 56: 54-67, 1906-07.
40. Pollak, L.: Kritisches und Experimentelles zur Klassifikation der Glykosurien, *Arch. f. exper. Path. u. Pharmacol.* 61: 376, 1909.
41. Luzzatto, R.: Die Glykosurie bei experimentellen Nephritiden, *Ztschr. f. exper. Path. u. Therap.* 16: 18, 1914.
42. Whitney, C.: Capillary Permeability in Acute Uranium Nephritis, *J. Path. & Bact.* 31: 699, 1928.
43. Vernon: These de Paris, 1885, cited by J. R. Lepine, *Le diabete sucre*, Paris, 1909, F. Alcan.
44. Kossa, J.: Ueber Chromsaure-Diabetes, *Arch. f. d. ges. Physiol.* 88: 627, 1902.
45. Kaiwa, T.: Hyperglycaemic Action of B-dimethyltelluronium Dichloride, *Tohoku, J. Exper. Med.* 20: 163, 1932.

ALLERGY IN RELATION TO PURPURA*

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SIR WILLIAM OSLER¹ in 1914 reported a series of purpura cases compiled over a period of nineteen years. He stressed the possible relationship of purpura with erythema multiforme or the erythema group, and pointed out the association of skin lesions and visceral symptoms. Alexander and Eyer-mann² in 1927 reported two patients in whom purpura was definitely related to the eating of various foods, as these patients were free from symptoms on the elimination of the incriminated food. In 1921 Duke³ reported a patient with severe abdominal pain associated with purpura and angioneurotic edema in whom there was a strong maternal and paternal familial history of purpura. Sensitization studies revealed marked reactions to a number of foods, and the purpura as well as the angioneurotic edema and abdominal complaints cleared up following a program of dietary restrictions. Peshkin and Miller,⁴ in their review of the literature, reported that purpura has been known to follow the administration of such drugs as mercury, aniline, iodine, pyridine, quinine, belladonna, ergot, acetphenetidin, salicylic acid, and arsphenamine.

Eyer-mann⁵ observed 18 cases in which purpura followed ingestion of certain foods. He stated that regardless whether the purpura and associated symptoms appear to be induced by infection, by ingestion, or by inhalation, the common factor appears to be a disorder of the capillary mechanism which leads to congestion, exudation, or hemorrhage. It may also lead to skin lesions, such as erythema multiforme, urticaria, angioneurotic edema, and purpura. In addition, he feels that exudations into the stomach or intestinal walls cause nausea, vomiting, and abdominal colic.

There are a number of other authors including Squier and Madison,⁶⁻⁸ Rowe,⁹ and Brown,¹⁰ who have contributed materially to the subject of purpura as related to allergy.

DISCUSSION OF ALLERGIC PURPURA

In a series of 64 patients with purpura hemorrhagica observed in the Cleveland Clinic, ten were thought to have a possible allergic factor related to their problem, and for this reason a complete allergy survey was done. The patients were analyzed from the viewpoint of the various purpuric manifestations, as well as for the correlation of the age incidence and sex.

There were four males and six females in the series. It was observed that the age factor did not play a prominent part, as there was an equal distribu-

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TABLE I

DISTRIBUTION OF VARIOUS ALLERGIC MANIFESTATIONS ASSOCIATED WITH PURPURA HEMORRHAGICA AS RELATED TO FAMILY HISTORY, BLOOD FINDINGS,*
AND OTHER ASSOCIATED CLINICAL MANIFESTATIONS

CASE NO.	ALLERGIC MANIFESTATIONS	FAMILY HISTORY	TOURNIQUET TEST	R.B.C. MILLIONS	Hb. %	W.B.C. THOUSANDS	COAGULATION TIME—MIN.	BLEEDING TIME—MIN.	CLOT RETRACTION MIN.	PLATELETS
1	Urticaria Gastrointestinal allergy	Positive	Not done	4.21	65	12.5	2	4	Normal	560,000
2	Urticaria Rhinitis	Positive	Positive	4.78	81	4.25	5	2½	Normal	100,000
3	Urticaria Rhinitis	Positive	Not done	4.87	75	6.25	10	3	Normal	310,000
4	Urticaria Gastrointestinal allergy	Positive	Not done	5.29	81	4.20				Normal
5	Urticaria Gastrointestinal allergy Rhinitis	Positive	Positive	4.20	84	6.20				
6a	Rhinitis (1)	Negative	Positive	5.00	81	10.70	6	7½	Normal	510,000
7b	Rhinitis Atopic dermatitis	Negative	Positive	5.15	78	10.75	5	4½	Normal	270,000
8	Gastrointestinal allergy	Positive	Not done	3.80	73	7.40	10	2	Normal	300,000
9c	Asthma	Positive	Negative	4.35	75	9.70		15†	Not done	40,000
10	Asthma Hay fever Urticaria	Positive Positive	Positive	4.70 4.68	71 71	13.5 5.65	12 10	2 20†	Normal None	Normal 20,000

*Normal averages as follows:

Coagulation time = 5 to 10 minutes.

Clot retraction = 1 to 2 hours.

Platelet count = 250,000 to 500,000.

Bleeding time = Up to 3 minutes.

a. Chronic nephritis; intestinal hemorrhage.

b. Chronic dental sepsis; acute nephritis; chronic prostatitis.

c. Essential hypertension; multiple myeloma; scurvy; ptosis of kidney.

tion of patients in the first, second, third, fourth, and seventh decades. Seven of the patients had tourniquet tests performed, and of this group five gave characteristic petechiae.

From the point of view of the different purpuric manifestations, 9 had petechiae, 7 had ecchymotic spots, 4 had recurrent attacks of epistaxis, 3 had bleeding gums, 2 had bloody stools, 2 had an abnormal catamenia, and one had had a cerebral hemorrhage.

PURPURA AND RELATED ALLERGIC MANIFESTATIONS

Table I shows the distribution of the various allergic manifestations associated with purpura hemorrhagica as related to family history, blood findings, and other associated clinical manifestations. There were only two patients who had a definite thrombopenia in addition to their allergy (see Cases 8 and 10). A detailed review of the ten cases follows:

CASE 1.—A boy, 2 years old, first visited the clinic in 1936. His mother stated that three months prior to admission, some abdominal rumbling with mucus and blood in the stools was noted. This cleared up after a week's duration, and was followed two days later by edema of the arms and legs and by purplish spots in the skin. Since that time he has had recurrent attacks of purpura with associated swelling of the legs. There had been considerable nasal congestion in addition to frequent colds. A bilateral family history of allergy was elicited. Sensitization studies were made, and the child was found to be allergic to a number of the more common foods and inhalants. His therapeutic program consisted of the avoidance of contact with inhalants and a program of dietary restriction. Three months later he was reported greatly improved without having had subsequent purpuric lesions and with a marked improvement of his nasal symptoms.

CASE 2.—A woman complained of recurrent hives for a period of five years. During the thirteen months prior to her admission, urticarial lesions had been present almost constantly. She had experienced recurrent exacerbations of the urticarial lesions following the ingestion of orange juice. Sensitization studies were made, and a program of avoidance or elimination of the reacting inhalants, foods, and molds controlled the urticarial lesions. Two years later, following a tonsillectomy, she had some bleeding from the rectum, nose, and gums, and an increased tendency to bleed easily. Tourniquet test was positive. Sensitization studies were repeated, but she did not materially improve on this program until after taking thyroid extract and Lugol's solution for a number of years.

CASE 3.—A woman, aged 32 years, was suffering from abdominal cramps, diarrhea, and mucus in the stools. She stated that certain foods, such as tomatoes, peaches, and cantaloupes, aggravated these symptoms. Further history revealed a perennial allergic rhinitis and a history of eczema. She had allergy studies at this time and was placed on a program of dietary restrictions and advised to avoid inhalants. Two weeks after returning home she developed purpuric symptoms about the right knee; these were thought to be due to some allergic factor that was not entirely controlled. The symptoms later cleared up satisfactorily.

CASE 4.—A woman, 65 years old, gave a history of recurrent purpuric spots for a period of twelve years prior to her examination. The lesions occurred mostly on the legs and thighs. There was a history of asthma which cleared up on a program of hyposensitization. She was of the opinion that nervous strain, sugars, pastries, and such foods as strawberries and tomatoes caused the purpuric spots to appear. She gave positive reactions to food, inhalant, and bacteria testing, and was placed on a restricted diet and instructed to avoid inhalants to which she reacted. When last seen, fourteen months after the institution of treatment, she reported that she had been getting along well and had had no recurrence of her purpura.

CASE 5.—A woman, aged 37 years, complained of a recurrent purpuric rash over her abdomen for the past several years. Eight weeks prior to admission she had observed this rash appearing over her arms and about her ankles. No known etiologic factors were elicited. She had had some stinging irritating lesions, presumably hives, following contact with hollyhocks, and on one occasion when changing feathers from one pillow to another. She gave a history of perennial rhinitis and frequent colds, especially during the winter months. She frequently experienced heartburn after eating white bread and pancakes. There was a definite family history of allergy. Physical examination revealed nothing of clinical significance other than the purpuric areas over her arms, thighs, and legs. Tourniquet test was positive and special blood examinations were within normal limits. She was placed on a restricted diet and told to avoid inhalants to which she reacted.

CASE 6.—A 16-year-old boy was first seen on March 3, 1939. He reported that in September, 1938, he worked in a new store for two days, a total of twenty-eight hours, during which time tar was being heated in the basement to repair the roof. The tar fumes were very strong during the entire period he was working. Within four or five days following exposure to the tar fumes, he developed severe cramps in his legs. This was followed by nausea and vomiting, and by black stools without diarrhea. Two weeks after his exposure he first developed reddish-purple spots of varying size on his arms, legs, and lower abdomen, and considerable edema of the feet. Within the next eight weeks (spent entirely in bed) the purpura was noted in successive crops. Hematuria was then discovered. On December 12, 1938, severe edema of the face became apparent, with the onset of more petechiae. On March 1, 1939, he again developed nausea and vomiting, with bloody diarrhea and hematuria, and the onset of a new crop of petechiae.

The boy had been having a chronic catarrh for many years which was worse during the winter months. He has noted definitely that being in contact with dogs and cats tended to make his nasal condition worse, and that the catarrh improved when he stayed away from them. Since September, 1938, hives have been noted at infrequent intervals. Eating of cooked cabbage, oatmeal, and several other cooked vegetables definitely caused nausea and vomiting. There was conclusive family history of allergy. The boy's paternal grandmother had chronic eczema, a paternal uncle has hay fever, and one paternal cousin has asthma. By further questioning, the fact was elicited that as a child the patient had chewed tar in large wads upon numerous occasions. Routine sensitization studies were performed which revealed very strong reactions to a number of the more common inhalants, molds and bacteria. Very strong reactions were likewise noted to a number of the more common foods and sea foods. Patch test to mild tar ointment in a lanolin base gave a slight erythema (patch test to lanolin alone was negative), while a patch test to crude tar ointment gave a very strongly positive reaction. The patient was placed on an allergy program consisting of dietary restriction, avoidance and hyposensitization with the positive inhalants to which he reacted. He was further advised to stop smoking entirely and to keep a food diary.

He was seen at weekly intervals for a period of six months and continued his program of hyposensitization during that period.

The urine examination of the patient revealed repeated specimens showing albumin varying from one to four plus, both granular and hyalin casts in varying numbers, and red and white blood cells. The entire picture showed a definite improvement, and the last several specimens showed only a trace of albumin, three to five red and white blood cells, and rare granular cast on microscopic examination.

A definite improvement in symptoms characterized by a feeling of general well-being, increased appetite, and a weight increase of 15 pounds after the first month, was observed. He continued for several weeks to have scattered purpuric lesions over the lower extremities, which gradually disappeared. He was last seen nine months after the treatment was instituted, and at that time his allergy was very well controlled. He had had no recurrence of his purpuric lesions. Urine examination, however, revealed few red blood cells. He has gained 50 pounds in weight since treatment was first started. We feel that he has a chronic nephritis.

CASE 7.—A man in his 60's gave a history of having had recurrent episodes of epistaxis, mainly during the winter months. Purpuric or ecchymotic spots were noted over the legs for a month prior to his visit to the clinic. He gave a history of recurring attacks of nasal obstruction, postnasal drip, and rhinorrhea for thirty years. The purpuric lesions were noted over the lower extremities and abdomen. He gave minimal reactions to the various allergens with the exception of bacterin, to which he gave very marked delayed reactions—more so than is usually encountered.

The patient's urine was examined at daily intervals during his period of hospitalization, which was of ten days' duration. Significant findings were albumin, varying from 1 to 3 plus. Both hyalin and granular casts were seen on microscopic examination with both red and white blood cells from zero to eight in number. There is no question of an acute nephritis in this case.

He was placed on a program of dietary restrictions and hyposensitization. He did not follow his program and we did not hear from him since outlining his treatment until recently, when his doctor reported his death due to chronic nephritis.

CASE 8.—A little girl, 2½ years old, had an atopic dermatitis showing the characteristic distribution. Dietary restriction cleared up the eczema satisfactorily without recurrence, but not the purpura. Since there had been a definite persistence of the purpuric lesions along with a thrombopenia, a splenectomy was performed. The patient is doing well with no recurrence of purpura over a three-month period, her platelet count being 90,000. Her purpura in retrospect was not related to allergy. The atopic dermatitis responded satisfactorily to treatment.

CASE 9.—A man, 30 years old, gave a history of recurrent crops of ecchymotic areas over his lower extremities for a period of five months. In addition, he stated that he had had asthma practically all his life. His only suggestion relative to the purpura was that he had been taking some white homeopathic pills which he had started about the time of the onset of symptoms. He further stated that he had had periods in which he was symptom-free, during the time that he was receiving the medicine. There was a very definite familial history of allergy. Physical examination revealed purpuric lesions over both lower extremities, with an associated scabies infection involving the trunk and groin.

There was some frank edema of the arms and legs, and his blood pressure was systolic 164 and diastolic 116 and later dropped to systolic 160 and diastolic 104. His urine showed a faint trace of albumin only on two occasions. Roentgenograms showed singular dense nodular areas in both lower lungs, and no definite etiology of this process could be determined. His eleventh and twelfth ribs revealed numerous tumor areas, and later he had pathologic fractures of these ribs. This was felt to be a possible multiple myeloma. Pyelograms were made, and the right kidney was found placed just below the rim of the pelvis. He received x-ray treatment over the ribs, and following this he showed some general improvement. Continued treatment over a period of months showed a definite improvement with a subsidence of the edema. It was very questionable as to whether his purpura was related to his allergy; however, the purpura cleared after several months.

Sensitization studies were done, and he was found allergic to a number of the common foods, inhalants, and molds. He was placed in a strict dietary regime, including the elimination of drugs, and instructed regarding the avoidance of inhalants. He was seen eighteen months after the original program of treatment was outlined and reported that he had been free of purpuric symptoms, and that he had observed a recurrence of his asthma only following the ingestion of milk.

CASE 10.—The other patient with a thrombopenia was a woman in her 20's who had definite seasonal (ragweed) hay fever and asthma, as well as urticaria and perennial rhinitis. She was placed on a program of pollen and inhalant hyposensitization. Her history revealed purpuric lesions for twelve years. She had an exacerbation of her purpuric symptoms following strenuous exercise, and on one occasion had a transient hemiplegia for several days. She did not follow recommendations regarding therapy, and her allergy symptoms have continued. Purpuric symptoms persisted with a low platelet count, and a splenectomy was advised. The splenectomy was performed and since then there has been no recurrence of purpura.

COMMENTS

The question of allergy has to be ruled out as an etiologic factor in purpura hemorrhagica. When a patient presents a frank allergy at the time of the purpuric lesions, such an allergy should be investigated and controlled if possible. When patients fail to respond to general therapeutic measures, even though there is no history of allergy, an allergy investigation is warranted, as well as in the case presenting only a family history of allergy.

Our study brings out very conclusively that an inhalant factor should be considered as a possible cause in some cases, and that when such a factor has been controlled the patient may respond to treatment as shown in Case 6.

It has been definitely proved that a program of dietary restrictions alone will control certain cases (Case 4).

SUMMARY AND CONCLUSIONS

1. Allergic manifestations, a personal history of allergy, or a family history of allergy warrants an allergy investigation as a possible etiologic factor in purpura hemorrhagica.

2. When all other methods of treatment have proved unsuccessful, an allergy investigation is warranted as a possible etiologic factor.

3. Of 64 patients with purpura hemorrhagica ten had complete allergy surveys as a possible allergic etiologic factor. Of these ten a positive family history of allergy was elicited from eight.

4. Thrombopenia was observed in two of the ten patients presenting a definite allergic problem.

5. One or more allergic manifestations were found to be present in each of the ten patients studied.

6. Petechiae were observed in nine patients and ecchymotic areas were observed in seven. These were the most frequent purpuric lesions demonstrated.

7. Two patients presented frank and common allergic manifestations but failed to show any improvement in their purpuric symptoms until after splenectomy. In both of these cases the platelet count was definitely reduced.

8. Chronic nephritis was present in two patients studied.

REFERENCES

1. Osler, Sir William: The Visceral Lesions of Purpura and Allied Conditions, Brit. M. J. 1: 517, 1914.
2. Alexander, H. L., and Eyermann, C. H.: Food Allergy in Henoch's Purpura, Arch. Dermat. & Syph. 16: 322, 1927.
3. Duke, W. W.: Food Allergy as a Cause of Abdominal Pain, Arch. Int. Med. 28: 151, 1921.
4. Peshkin, M. M., and Miller, J. A.: Quinine and Ergot Allergy and Thrombocytopenic Purpura, J. A. M. A. 102: 1737, 1934.
5. Eyermann, C. H.: Allergic Purpura, South. M. J. 28: 341, 1935.
6. Squier, T. L., and Madison, F. W.: Thrombocytopenic Purpura Due to Food Allergy, J. Allergy 8: 143, 1937.
7. Madison, F. W., and Squier, T. L.: Etiologic Factors in Hemorrhagic States, Wisconsin M. J. 37: 636, 1938.
8. Madison, F. W., and Squier, T. L.: Leukothrombopenia With Increased Capillary Permeability, J. A. M. A. 112: 879, 1939.
9. Rowe, A. H.: Clinical Allergy Due to Foods, Inhalants, Contactants, Fungi, Bacteria and Other Causes: Manifestations, Diagnosis and Treatment, Philadelphia, 1937, Lea & Febiger, pp. 469-473.
10. Brown, O. H.: Purpura Hemorrhagica From Food Sensitization; Successful Treatment by Dietary Regulation and Use of Digestants; Case Report, Southwestern Med. J. 19: 131, 1935.

EFFECT OF SULFAPYRIDINE ON STAPHYLOCOCCUS TOXIN*

AN IN VIVO STUDY IN THE RABBIT

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CARPENTER and Barbour¹ observed that staphylococcus toxin was inactivated in vitro by sulfanilamide. They found that 92 per cent of 630 mice survived when injected intra-abdominally with a sulfanilamide-staphylococcus toxin mixture. All 195 controls died when given toxin alone. These investigators also noted that the in vivo action of staphylococcus toxin was less marked when sulfanilamide was given either before or subsequent to the toxin. Carpenter, Barbour, and Hawley² likewise found that 89 per cent of 400 mice injected intraperitoneally with a sulfanilamide-staphylococcus toxin mixture survived while all of a group of 100 controls that received only the toxin died.

Rigdon and Freeman³ observed that sulfapyridine had no effect on the hemolytic, the skin necrotizing, and the lethal factors in staphylococcus toxin. They did note, however, a bacteriostatic action produced by this drug on staphylococci in vitro and also a variation in the degree of the action of sulfapyridine on three different strains of staphylococci.

The effect of sulfapyridine on the lethal action of staphylococcus toxin is studied in the present experiment.

MATERIALS AND METHODS

Fifteen cubic centimeters of a 25 per cent solution of sulfapyridine† in a 50 per cent dextrose solution were injected into the marginal ear veins of 28 normal adult rabbits. A similar quantity of 50 per cent dextrose was given to 18 animals. Five-tenths gram of sodium sulfapyridine in 5.0 c.c. of saline was injected intravenously into each of five rabbits. Thirty minutes following the injection of the sulfapyridine each rabbit was given either 0.15 c.c. or 0.3 c.c. of staphylococcus toxin intravenously. For one of the control observations 34 rabbits were given a corresponding amount of staphylococcus toxin. For a second control 10 rabbits were given 2,800 units of staphylococcus antitoxin intravenously and thirty minutes later 0.3 c.c. of staphylococcus toxin. The time of death was recorded. Animals surviving for forty-eight hours or longer were considered protected against the lethal effects of this toxin.

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†The sulfapyridine in dextrose and the staphylococcus toxin and antitoxin were supplied by Lederle Laboratories. Merck & Co. supplied the sodium sulfapyridine.

The amount of free sulfapyridine in the blood was determined by the method of Marshall and Litchfield.⁴ Blood was removed from the heart at the following intervals: thirty minutes, and one, two, three, five, and eight hours. Groups of four rabbits were bled at alternate intervals. The average sulfapyridine determination was used to establish the points on the curve (Chart 1).

TABLE I
EFFECT OF SULFAPYRIDINE ON LETHAL ACTION OF STAPHYLOCOCCUS TOXIN
(All injections were made intravenously.)

NUMBER OF RABBITS	SULFAPYRIDINE	AMOUNT OF STAPHYLOCOCCUS TOXIN (C.C.)	AVERAGE WT. (GM.)	NO. AND TIME* OF DEATH FOLLOWING INJECTION OF TOXIN
24		0.3	2,030	Group A 19 Group B 2 Group C 1 Survived 2
20	15 c.c. of 25% sulfapyridine in 50% dextrose	0.3	2,578	Group A 14 Group B 5 Group C 0 Survived 1
13	15 c.c. of 50% dextrose	0.3	2,493	Group A 3 Group B 4 Group C 1 Survived 5
10		0.15	1,334	Group A 3 Group B 3 Group C 2 Survived 2
8	15 c.c. of 25% sulfapyridine in 50% dextrose	0.15	1,315	Group A 0 Group B 3 Group C 4 Survived 1
5	15 c.c. of 50% dextrose	0.15	1,558	Group A 1 Group B 2 Group C 1 Survived 1
5	0.5 Gm. of sodium sulfapyridine in 5 c.c. of saline	0.15	1,390	Group A 1 Group B 4 Group C 0 Survived 0
10	2,800 units of staphylococcus antitoxin	0.3	1,870	Group A 0 Group B 0 Group C 0 Survived 10

*Group A—Rabbits dying from 0-2 hours following injection of the toxin.
Group B—Rabbits dying from 2-12 hours following injection of the toxin.
Group C—Rabbits dying from 12-48 hours following injection of the toxin.

EXPERIMENTAL

The number of rabbits used in each group, the amount of sulfapyridine in dextrose, dextrose alone, sodium sulfapyridine in saline, staphylococcus antitoxin, and staphylococcus toxin, and the number of rabbits that died at the different intervals are shown in Table I. It is evident from these data that neither sulfapyridine in dextrose nor sodium sulfapyridine in saline prevents death produced by an intravenous injection of staphylococcus toxin. A total of 2,800 units of staphylococcus antitoxin, given thirty minutes before the lethal dose of toxin, completely protects rabbits against the toxin.

Chart 1 shows the concentration of free sulfapyridine in the blood after an injection of sulfapyridine in dextrose. The rate of excretion appears to be very rapid. The free sulfapyridine in the blood at specific intervals after an intravenous injection of 0.5 Gm. of sodium sulfapyridine in saline is as follows: thirty minutes, 13.2 mg. per cent; one hour, 13.3 mg.; two hours, 9.2 mg.; three hours, 4.46 mg.; and five hours, 2.4 mg.

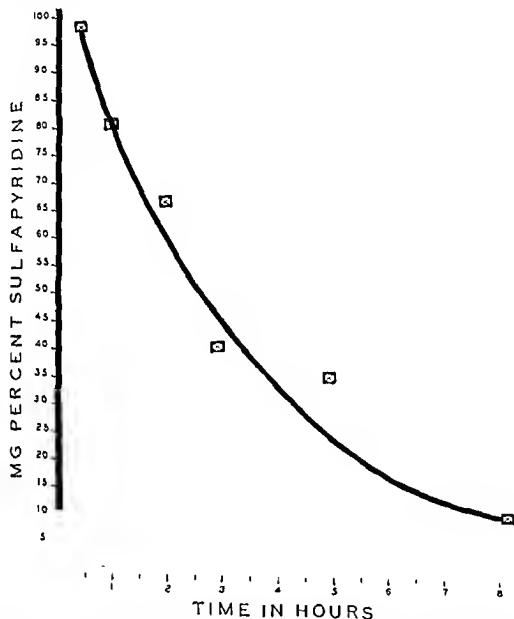


Chart 1.—Amount of free sulfapyridine in the blood of rabbits following an intravenous injection of 15 c.c. of a 25 per cent solution of sulfapyridine in 50 per cent dextrose.

It is interesting to observe in Table I that rabbits given 15 c.c. of a 50 per cent solution of dextrose and 0.3 c.c. of staphylococcus toxin thirty minutes later, live for a longer time than those animals given either the sulfapyridine in a similar quantity of dextrose and 0.3 c.c. of staphylococcus toxin or the toxin only. The rabbits given both the sulfapyridine in dextrose and only the dextrose bled very freely from the venous puncture following each injection. There was no difference in either the bleeding or the clotting time of these rabbits when compared with the controls.

Two rabbits died while they were being injected with the sulfapyridine in dextrose. One died while receiving the dextrose. The ear in which both the sulfapyridine in dextrose and the dextrose were injected became edematous after several minutes. Extensive necrosis of the ears usually occurred in those rabbits that survived the lethal action of the toxin.

DISCUSSION

The data presented here show that sulfapyridine has no inhibitory effect on the lethal action of staphylococcus toxin when given intravenously to the rabbit. Taylor and his associates⁸ are of the opinion that when sulfapyridine is given in dextrose to man the drug circulates in the blood in a form different from other sulfapyridine preparations. If such occurs in the rabbit, one would then conclude from these experiments that it also has no effect on the lethal action of staphylococcus toxin.

There is a significant difference in the twelve-hour survival rate of the rabbits given dextrose and 0.3 c.c. of staphylococcus toxin over those injected with only the toxin. This difference is two and one-half times its standard error. A larger number of rabbits given dextrose and 0.3 c.c. of staphylococcus toxin lived more than two hours longer than those animals injected with sulfapyridine in dextrose and a similar quantity of toxin. There is no difference in the low hour survival rate, however, in the rabbits given sulfapyridine in dextrose and toxin over those animals given only the staphylococcus toxin.

The failure of the rabbits that received the sulfapyridine in dextrose to survive for a longer time than the rabbits given only the toxin suggests that the effect of the sulfapyridine, plus that of the toxin, is sufficient to produce death more quickly than the toxin alone. A phenomenon similar to this was observed by one of us (R. H. R.) in mice given sulfapyridine and staphylococcus toxin. Eighteen mice in a group of 30 died when a mixture of sulfapyridine and toxin was given intraperitoneally, while only 15 died in a group of 30 when given only the staphylococcus toxin.

The results of this experiment suggest that the hypertonic dextrose solutions may inhibit the union of staphylococcus toxin with tissue cells. One of us (R. H. R.) recently studied hemolysis produced by staphylococcus toxin on rabbit red blood cells suspended in hypertonic solution of sodium chloride and found an inhibition in the rate and the degrees of lysis which paralleled the concentration of the salt.⁹ In another study it was observed that necrosis failed to occur at the site of intradermal injection of staphylococcus toxin when a 5 per cent solution of sodium chloride was previously injected into the local area of the rabbits' skin. Necrosis occurred in these rabbits below the point of injection of the toxin. It was thought that "the inhibitory action of sodium chloride on the dermal necrosis produced by staphylococcus may be the result of the action of the salt on the tissue cells. The change effected may inhibit the union of the toxin with the cells. This union apparently is responsible for the necrosis."

Smith⁷ has observed that sucrose, glucose, glycerol, and ethylene glycol inhibit the necrotic action of staphylococcus toxin. This inhibition appears to be related to the hydroxyl content rather than to the molecular weight. The mechanism of the action is one of partial destruction of the toxin. The staphylococcus toxin in our experiment must not have been destroyed, since the total number of deaths within a period of forty-eight hours in the group of rabbits receiving the dextrose and the toxin and the group receiving only the toxin show no significant difference.

It appears that the rate of excretion of sulfapyridine in dextrose following an intravenous injection in the rabbit is similar to that which occurs in man.⁸

The results of this experiment suggest that sulfapyridine would have no effect on the toxin liberated by certain strains of staphylococci in vivo. The pathologic lesions caused by toxin-producing strains of staphylococci in vivo are similar to those lesions produced by staphylococcus toxin which was prepared in vitro and then injected locally into experimental animal.^{9, 10} Any effect that sulfapyridine might have on a staphylococcus infection apparently would be other than by its action on the toxin.

CONCLUSIONS

Sulfapyridine does not inhibit the lethal action of staphylococcus toxin in the rabbit.

Rabbits given an intravenous injection of 50 per cent dextrose and thirty minutes later staphylococcus toxin survive for a longer time than those animals given only staphylococcus toxin.

REFERENCES

1. Carpenter, C. M., and Barbour, G. M.: Inactivation of Toxins of *Staphylococcus aureus* and *Clostridium welchii* in Vitro by sulfanilamide, *Proc. Soc. Exper. Biol. & Med.* 41: 354, 1939.
2. Carpenter, C. M., Barbour, G. M., and Hawley, P. S.: The Inactivation of Staphylococcal Toxin in Vitro by Sulfanilamide, *J. Pediat.* 14: 116, 1939.
3. Rigdon, R. H., and Freeman, P.: The Effect of Sulfapyridine on Staphylococci and Staphylococcus Toxin, *J. LAB. & CLIN. MED.* 25: 1125, 1940.
4. Marshall, E. K., and Litchfield, J. T.: The Determination of Sulfanilamide, *Science* 88: 85, 1938.
5. Rigdon, R. H.: Inhibitory Effect of Hypertonic Solutions of Sodium Chloride on Staphylococcus Hematoxin, *J. Infect. Dis.* 60: 25, 1937.
6. Rigdon, R. H.: Inhibitory Action of Sodium Chloride on Skin Necrosis Produced by Staphylococcus Toxin, *Arch. Path.* 22: 763, 1936.
7. Smith, M. L.: The Influence of Glycerol on the Skin Necrosis Produced by Staphylococcus Toxin, *Brit. J. Exper. Path.* 18: 265, 1937.
8. Taylor, F. H. Laskey, Lowell, Francis C., Adams, Margaret A., Spring, William C., and Finland, Maxwell: A Comparative Study of the Blood Concentrations and Urinary Excretion of Sulfapyridine and Sulfanilamide After Single Doses of Sulfapyridine and Related Compounds Administered by Various Routes, *J. Clin. Investigation* 19: 201, 1940.
9. Rigdon, R. H.: Local Lesions Produced in Mice by Staphylococcus Toxin and by Toxin and Non-Toxin Producing Strains, *Surgery* 6: 91, 1939.
10. Rigdon, R. H.: Hemolysis Produced by Staphylococcus Colonies and Toxin on Agar Media Containing Various Animal Bloods, *J. LAB. & CLIN. MED.* 24: 1264, 1939.

ELECTROCARDIOGRAPHIC CHANGES FOLLOWING THE INTRAVENOUS ADMINISTRATION OF MAGNESIUM SULFATE*

III. COMBINED EFFECT WITH DIGITALIS

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IT HAS been previously shown by means of the electrocardiogram that magnesium when given intravenously has a definite effect on the cardiac conductive system.¹ This effect consists of an early acceleration followed by slowing, with delay in the atrioventricular and intraventricular conduction time and increased excursion of all complexes. It has been shown that this action of magnesium upon the conductive system is not central but direct.²

Zwilling^{3, 4} showed that magnesium had a beneficial but temporary effect on digitalis intoxication. He found it useful in combating ectopic impulses from any source, paroxysmal tachycardia, ventricular fibrillation and heart block. He believed that magnesium had no untoward effect on an already damaged conduction system. He was able to save rabbits who had been given lethal doses of digitalis with repeated small doses of magnesium. All control animals died. Similar studies were done on frogs. Block and Pick⁵ confirmed the clinical studies of Zwilling, citing a single case. Smith, Winkler, and Hoff⁶ concluded from their studies on magnesium that Zwilling's work has little physiologic support since such arrhythmias develop spontaneously or can be evoked in the presence of a greatly increased concentration of magnesium in the serum.

The present problem was undertaken to ascertain the effect of magnesium sulfate, as measured by the electrocardiogram, on the conductive system of the digitalized dog.

METHOD

As in previous experiments^{1, 2} the unanesthetized dog was placed on the left side and electrodes were attached to the right front and left rear legs, thus corresponding to the conventional second lead. Electrocardiograms were made on a string galvanometer type machine. Both magnesium sulfate and digitalis were injected into the right saphenous vein.

EXPERIMENTS

Each dog underwent three separate experiments at no less than weekly intervals. These consisted of (1) the administration of magnesium sulfate, (2) digitalis, and (3) digitalis followed in twenty minutes by magnesium.

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Experiment 1.—Magnesium was administered intravenously, and the results obtained were in agreement with those previously reported.

Dog 1, weight 13.6 kg., received 10 c.c. of a 20 per cent magnesium sulfate solution given at the rate of 2 c.c. per minute (Fig. 1). The rate was at first accelerated following by slowing. The P-R interval increased from 0.16 to 0.18 second, and the QRS interval from 0.04 to 0.06 second. The T-waves originally inverted became diphasic.

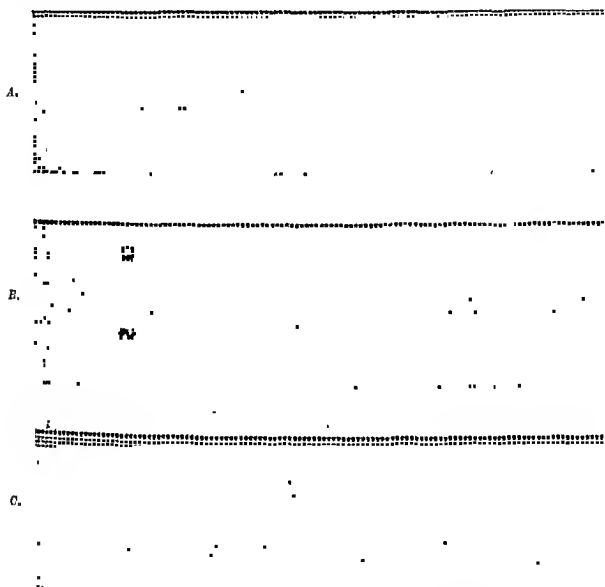


Fig. 1.—Dog 1. A, Normal graph. B, Following the injection of magnesium sulfate. Note increase in rate (130). C, Twenty minutes after injection.

Dog 2, weight 20.5 kg., received the same amount of magnesium sulfate. The changes in rate were not unlike those of Dog 1. The P-R interval increased from 0.14 to 0.18 second, and the QRS interval from 0.05 to 0.07 second.

Experiment 2.—In this experiment digitalis (Digalen) was given intravenously as rapidly as possible. It was found that approximately $1\frac{1}{3}$ eat units per kilogram of body weight were sufficient to produce definite electrocardiographic changes. These changes consisted of bradycardia, slowing of the auriculoventricular and ventricular conduction times, and increased excursion of the T-wave. The only objective evidence of toxicity was vomiting, although as much as 28 eat units were given intravenously.

Dog 1 received 20 eat units of digitalis (Digalen). The rate fell from 80 to 60 beats per minute (Fig. 2). The P-R and QRS intervals increased from

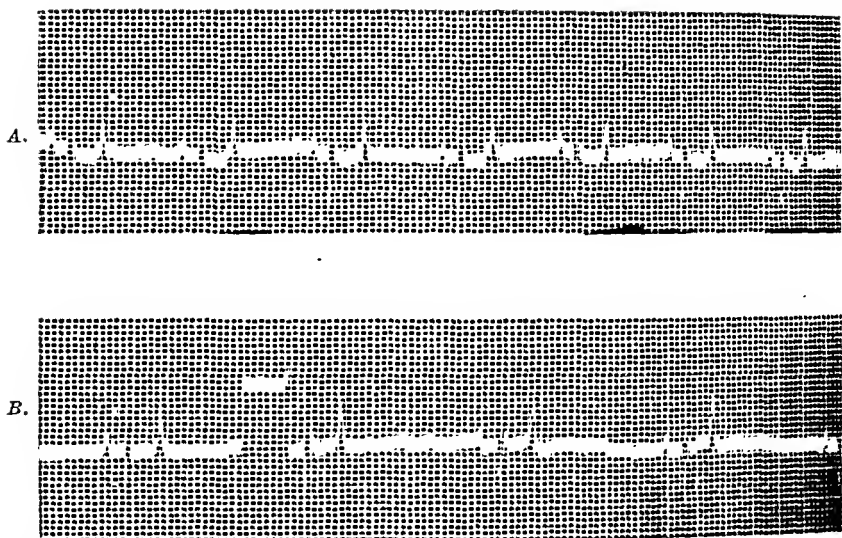


Fig. 2.—Dog 1. *A*, Normal graph. *B*, Twenty minutes after the intravenous administration of 20 cat units of digitalis (Digalen). Note the bradycardia, increased P-R interval, and elevation of the T-wave.

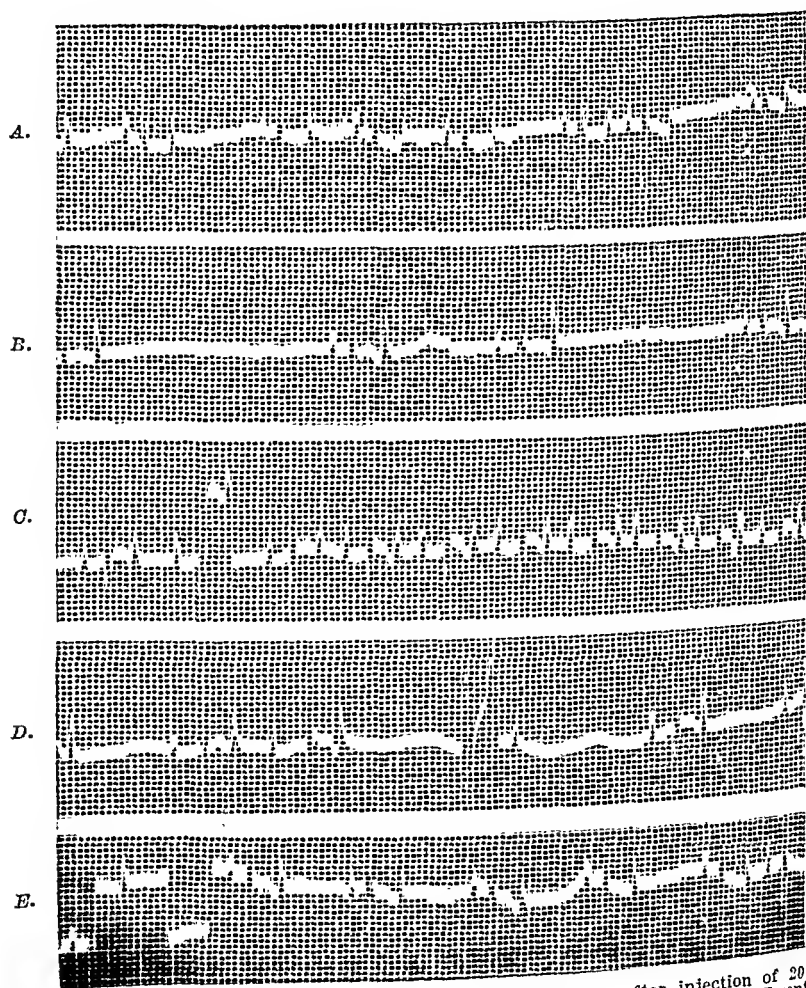


Fig. 3.—Dog 1. *A*, Normal graph. *B*, Twenty minutes after injection of 20 cat units digitalis (Digalen). *C*, Following injection of magnesium sulfate solution. *D*, Twenty minutes later. P-R interval now 0.20 second. *E*, Eighteen hours from beginning of experiment.

0.11 to 0.13 second and 0.04 to 0.05 second, respectively. The T-wave became more upright by 100 per cent. These changes were still present at the end of twenty-five minutes, at which time the experiment was terminated.

Dog 2 received 28 cat units of digitalis. The P-R interval increased from 0.16 to 0.18 second, but the QRS complexes were unaffected. The T-wave became more sharply inverted. A marked sinus arrhythmia occurred.

Experiment 3.—The same amount of digitalis was then given to each animal as in previous experiments, followed in twenty minutes by the same amount of magnesium sulfate. Following the administration of digitalis, tracings similar to those in Experiment 2 were obtained.

After the administration of magnesium the rate in Dog 1 increased from 40 to 100 beats per minute, following which it became slower, but at no time did it approach the bradycardia attained with magnesium only (Fig. 3). The P-R interval, which had been 0.12 second at the beginning of the experiment, increased to 0.14 second following administration of digitalis, and to 0.20 second following magnesium. The latter auriculoventricular conduction time is far in excess to that obtained in Experiments 1 and 2. A marked sinus arrhythmia which had developed following the administration of digitalis almost disappeared following the administration of magnesium; however, numerous premature ventricular contractions were now present. An electrocardiogram made eighteen hours later was not unlike that seen at the beginning of the experiment, except for a slight prolongation of the P-R interval most likely of digitalis origin.

When magnesium was administered to Dog 2, the P-R interval, which was originally 0.16 second and had been elevated to 0.18 by digitalis, was increased to 0.28 second. Instead of the sinus arrhythmia which followed the administration of digitalis, a 2:1 heart block developed.

In Dog 3 magnesium not only failed to control a 2:1 heart block produced by digitalis, but also prolonged the auriculoventricular conduction time from 0.16 to 0.22 second, and the ventricular conduction time from 0.06 to 0.07 second. In Dog 4 digitalis produced an occasional 2:1 heart block, but following the administration of magnesium a continuous block developed. The T-waves became sharply inverted, and the auriculoventricular conduction time increased from 0.20 to 0.22 second.

These results do not agree with the clinical reports of Zwillinger. Magnesium sulfate given to the digitalized dog failed to regulate the sinus arrhythmia and actually provoked the occurrence of premature beats and heart block. Magnesium sulfate is also capable of prolonging the auricular conduction time in excess of that resulting from digitalis or magnesium alone. An initial increase in rate was noted, followed by slowing, but the rate was more rapid than that produced by digitalis. This apparently demonstrates that magnesium overcomes the vagal stimulation of digitalis.

CONCLUSIONS

1. In the digitalized dog magnesium sulfate is capable of altering the contour of the T-waves and producing a further delay in auriculoventricular conduction time in excess of that of digitalis or magnesium alone.

2. In the digitalized dog magnesium sulfate causes a brief increase in rate, followed by a slowing which approaches that obtained by digitalis. It is thus capable of overcoming the vagal stimulation produced by digitalis but for short periods only.

3. In the digitalized dog magnesium sulfate is capable of producing impulses of ectopic origin.

4. The results indicate that magnesium sulfate does not overcome the effects of digitalis intoxication but increases the degree of block and the occurrence of ectopic impulses.

REFERENCES

1. Miller, J. Roseoc, and Van Dellen, T. R.: Electrocardiographic Changes Following the Intravenous Administration of Magnesium Sulfate. An Experimental Study on Dogs, *J. LAB. & CLIN. MED.* 23: 914, 1938.
2. Van Dellen, T. R., and Miller, J. R.: Electrocardiographic Changes Following the Intravenous Administration of Magnesium Sulfate. II. An Experimental Study on Dogs. *J. LAB. & CLIN. MED.* 24: 840, 1939.
3. Zwillinger, L.: The Effect of Magnesium on the Heart, *Klin. Wehnschr.* 14: 1420, 1935.
4. Zwillinger, L.: The Effect of Magnesium Sulfate on Cardiac Intoxication Due to Strophanthin (Strophanthus Preparation), *Wien. klin. Wehnschr.* 49: 594, 1936.
5. Block, C., and Pick, A.: The Effect of Magnesium in Digitalis Intoxication. Clinical Study of : : : : : Ventricular Rhythm of Stimulus, *Wien. Arch. f. inn. Med.* 29: 435, 1936.
6. Smith, P. K., Winkler, A. W., and Hoff, H. E.: Electrocardiographic Changes and Concentration of Magnesium in Serum Following Intravenous Injection of Magnesium Salts, *Am. J. Physiol.* 126: 720, 1939.

COMBINATIONS OF LEAD, ARSENIC, AND OTHER CHEMICALS WITH EXPERIMENTAL ASPHYXIA OF TUMORS

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OTHER papers¹ have described the effects of prolonged local asphyxia of normal parts and also of tumors, especially the transplantable carcinomas and sarcomas of rats and mice. The most striking effects on tumors are (a) dark hemorrhagic congestion of the tumor in contrast to the bright arterial hyperemia of the normal tissues of the limb after ligation, and (b) extensive necrosis of the tumors, sometimes to the point of complete cure, without permanent damage to the normal tissues. Subsequent papers² will describe similar powerful effects of temporary asphyxia on spontaneous tumors, including the complete cure of one patient with cancer of the skin. While the ligation method is necessarily unsuited for any extensive use in practical tumor treatment, there is experimental evidence² of its ability to augment the effect of x-ray treatment. Other attempted combinations have been less promising, but even the negative results appear worth recording for the sake of theoretical information on the tumor problem.

Chemotherapy might be advanced by the finding of a substance which would show at least a partial selectivity for tumor tissue, and which by acting as an adjuvant to asphyxia would make the destruction of tumors safer and more

efficient. According to the theory of Neuberg and Caspari,³ the hyperemia following ligation should strengthen the chemotherapeutic action by carrying systemically injected substances in larger proportion to the tumor area and in smaller proportion to other parts of the body. For locally injected drugs there is the advantage of lying for several hours in direct contact with the tumor, with diffusion perhaps aided by altered permeability and asphyxial injury of the tumor cells.

ARSENIC

Arsenic in topical applications has been used to cure some tumors since the time of the Egyptians⁴; it is also capable of producing tumors.^{5, 6} Its pharmacology is of particular interest in the present connection. Cushman⁷ states that "arsenic is a capillary poison, dilating capillaries and permitting edema formation more readily than the normal. Arsenites and arsenious acid do not coagulate proteins or change them in any way. . . . It seems to act only upon living cells, and unlike acids and alkalis, forms no combination with the dead tissues."

Sollmann⁸ says, "Arsenical compounds act locally as mild and slow corrosives, and have been employed in superficial cancer. Systemically, they relax the capillaries and increase their permeability, thus stimulating inflammation." Arsenic is said to be toxic to all animals with a central nervous system, and to some lower forms, but it cannot be classed as a general protoplasmic poison. It has little effect on ferments, although "it is now generally believed that the arsenicals hinder protoplasmic oxidation in an unknown way." Arsenic is described as causing peripheral vasodilatation and paralysis of capillaries. "This view is favored by the fact that they have become more permeable. Intravenous injections of large quantities of salt solution will cause edema in animals poisoned with arsenic, but not in the normal. Since increased permeability of the capillaries is one of the essential features of inflammation, the phenomena of arsenic poisoning are similar to those produced by an irritating inflammation, although the primal cause is different. Arsenic produces nephritis, at first mainly vascular, but always with more or less inflammation of the epithelium." These statements, are supported by references to the well-known works of Magnus, MacNider, and others.

Sollmann further states: "Arsenicals are not precipitant and therefore irritate only weakly, even on wounds and mucous membranes, but the cells die slowly after prolonged contact. They are sometimes used for killing dental nerves, and as salves for superficial epitheliomas. Arsenic is much more destructive to pathologic cells, so that the cancerous tissue may be killed without injury to the surrounding normal tissue. As with all cancer salves, some of the deeper tumor cells usually escape, leading to inoperable recurrences."

Because the effects of arsenic so closely resemble those of asphyxia, arsenic was selected for a long series of systemic and constitutional treatments of rat tumors. Beginning with the pharmacopeial Fowler's solution, containing 1 per cent arsenic trioxide, decimal dilutions were made with physiologic saline to contain from 1 mg. to 0.00001 mg. per cubic centimeter.

Systemic Treatment.—Willberg's⁹ scale of arsenic tolerance of different species omits the rat. Experience has indicated that the lethal dose is near 1 mg.

per 100 Gm. of weight, with slight variations according to age, obesity, etc., but much more according to general strength. As weak animals have a decidedly reduced arsenic tolerance, animals with large tumors or with ligation treatment demand smaller doses than the normal. Those in best condition could receive as high as 0.9 mg. per 100 Gm., but the usual doses were smaller. Acute death from arsenic has nothing to do with organic lesions in the liver, kidneys, or elsewhere. In harmony with the above-mentioned pharmacologic action of arsenic, the picture resembles shock, and acute arsenic poisoning is perhaps correctly classified under shock. At least in the rat, the elimination is evidently rapid and the danger of cumulative effects is slight. Thus, even tumor-bearing rats were able to receive doses of 0.6 or 0.7 mg. twice or sometimes three times in twenty-four hours and to continue this sometimes more than two weeks without serious symptoms. Feeding the drug was not attempted. Subcutaneous injections produce slow ulcerations. Therefore intraperitoneal injections were usually employed.

The tumor experiments may be summarized as follows: Positive results were obtainable with single arsenic injections followed by single ligations, inasmuch as sloughing of all visible tumor tissue followed ligations of only two or three hours, which is never possible with ligation alone. Similar effects were obtained with various combinations of repeated arsenic injections and repeated ligations. A few cures were positive, in the sense of indefinite survival without tumor recurrence. But with all kinds of combinations having any decisive effect upon the tumors, the mortality was overwhelmingly high, as has been the usual rule with all chemotherapeutic attempts in the literature. Inasmuch as a lower mortality and a higher proportion of cures were obtainable with ligation alone, the combination with systemic arsenic administration did not appear promising.

Similar experiments with single and repeated arsenic injections were performed with the chicken sarcoma. Willberg⁹ gives the tolerance dose of arsenic for chickens as 0.06 Gm. per kilogram. The single doses in tumor-bearing chickens ranged only from 5 to 7 mg., but these were sometimes continued for as long as nine days. Results were positive only to the extent that sloughing of tumors after a given duration of ligation was more extensive with the arsenic injections than without. The general results were inferior to those in rats, and no cures were obtained.

Local Arsenic Injections.—Although arsenic produces remarkably little irritation in the normal tissues, it may, as already mentioned, give rise to ulcers when injected subcutaneously. As should naturally be expected, the necrotizing effect is increased by asphyxia. An approximate scale of tolerance was worked out in normal rats for combinations of one to five hours of asphyxia with arsenic dilutions from 10 mg. to 0.0001 mg. per cubic centimeter; for example, the undiluted Fowler's solution (10 mg. per cubic centimeter) caused ulceration with one hour of ligation and fatal gangrene with two hours. The concentration of 1 mg. per cubic centimeter was compatible with ligation to the limit of three hours, but caused gangrene with four hours. The solution of 0.1 mg. per cubic centimeter and weaker dilutions could be tolerated with ligations of four and

five hours. A modifying factor was later encountered, in that the quantity or location of the injection may create an increased pressure in the tissues, which, if continued, increases the tendency to gangrene.

The results with tumors may be summarized as follows: Without ligation, rat tumors are not destroyed by single local arsenic injections in any of the concentrations mentioned, or by fractional quantities infiltrated every fifteen minutes as as to keep the tumor thoroughly bathed in the solution for three or four hours, even if epinephrine or ephedrine is added. With any length of ligation, an arsenic dilution of 0.00001 mg. per cubic centimeter resembles plain saline solution in showing no perceptible effect, but all stronger solutions had an influence in destroying tumors. Cures of sarcoma 39 were obtained with ligations of two to three hours preceded by injections ranging from 0.2 c.c. of 10 mg. arsenic per cubic centimeter to 5 c.c. of 0.0001 mg. per cubic centimeter solution. In successful cases the arsenic not only shortened the time of ligation but also sharpened the specificity; the tumors were killed with less damage to the normal tissues than from ligation alone, and the resulting ulcers healed much more rapidly. Statistics are meaningless because success appeared to be governed entirely by one mechanical condition. The rat tumors are firmer in consistency than the surrounding normal tissues, so that a satisfactory infiltration of the tumor is difficult to obtain and the fluid tends to accumulate particularly in the loose subcutaneous tissue. If pressure is used to compel penetration, the normal tissues become subject to gangrene. If the first treatment is only partially successful, a repetition is practically impossible, because the injection fluid cannot be retained in an open ulcerating tumor, though collodion coating and other devices were used in the attempt. For this reason, the permanent cures, though individually rather spectacular, were comparatively few among the sarcomas and were absent with the Flexner-Jobling carcinoma and the Walker tumor.

For the same mechanical reason, attempts to combine ligation and arsenic injection in the treatment of spleen, kidney, and other intra-abdominal tumors all failed. On the other hand, a small series of serotal and testicular sarcomas turned out favorably. The testis offers a special condition because of its firm tunics, and the strong reaction of the serotum to irritation (as mentioned previously under ligation alone)¹ perhaps conduced to the cures which were obtained, with ligations of thirty to ninety minutes preceded by injection of 0.5 to 1 c.c. solution of 0.01 to 0.1 mg. arsenic per cubic centimeter. In all these cases, however, the testes became atrophied and the animals were sterile in breeding tests. Therefore, a cure with selective preservation of the testis was not obtained.

A small series of mice, with sarcoma 180 of 0.8 cm. diameter in the legs, were treated by injection of the tumors with 0.5 c.c. solution containing 0.001 mg. arsenic per cubic centimeter, followed by ligation for twenty, forty, sixty, eighty, and one hundred minutes, respectively. The tumors ligated for twenty and forty minutes merely ulcerated; the others were permanently cured. A single trial of forty minutes' ligation preceded by infiltration with 0.5 c.c. of a stronger arsenic solution (0.01 mg. per cubic centimeter) resulted in a cure.

Arsenic injections combined with ligation of the chicken sarcoma contributed to the breakdown of the tumor tissue but in no case led to a cure.

LEAD

The phenomena described by Bell¹⁰ and others, of swelling, hyperemia, and more or less necrosis in tumors under lead treatment are suggestively similar to the results of ligation. Although it is the consensus of later work that clinical cures are not thus obtained, the use of local asphyxia to reinforce the systemic lead administration appeared attractive. Thanks are due to Dr. William H. Kraemer for supplying his preparations of colloidal lead phosphate on two occasions, the first time with a small admixture of manganese, and the second time plain.

A series of 39 rats, with medium or large tumors, were not able to tolerate as high dosage as described by Kraemer.¹¹ The stronger animals, of 150 to 200 Gm. weight, often died from intravenous injections above 1.5 c.c. of the suspension, containing 4 mg. of metallic lead per cubic centimeter. For all animals it was found safest to divide doses of 1 c.c. or more into two equal injections, morning and evening. Even with these precautions, administration at the rate of about 2 mg. of lead per 100 Gm. weight daily was never tolerated for more than two or three days, and rats commonly died within a few days after the last injection. The explanation may lie in the relation between the general strength and resistance to lead. The tumor rats may have had some impairment of vigor even when they appeared in good condition, and when they were perceptibly weak or anemic their tolerance of lead was much lower.

The tumors were not perceptibly affected by small doses of lead within the limits of safety. With dangerously large or fatal doses the sarcoma and carcinoma (but not the Walker tumor) sometimes showed softening and more or less necrosis, but there was no instance of cure with survival.

When the tumor-bearing legs were ligated for periods of one to three hours in connection with the lead treatment, there were indications of an increase of necrosis in the tumors without increase of damage to the normal leg tissues. In cases where the rats survived, this necrosis appeared so complete as to give promise of cure. The purpose had been to achieve a cumulative effect of the lead and ligation upon the tumors. The dominant result was actually a cumulative effect upon the animals, the ligated ones dying from doses which could be tolerated by nonligated controls. No cures were obtained.

A few attempts were also made to combine the lead treatment with intraperitoneal or intravenous arsenic injections, given during a different time in the same day, in the hope that a nontoxic dose of arsenic might have an adjuvant action with a nontoxic dose of lead to destroy a tumor. The same sort of failure resulted, because one poison lowered the tolerance of the animal to the other.

Remarks in the literature, attributing the deaths of lead-treated rats to anemia, are erroneous. Numerous additional experiments were performed, in which single or repeated transfusions were given before or after the lead treatment, or the lead suspension was mixed with fresh heparinized rat blood for injection. It is a simple matter by these means to overcome anemia entirely even in animals which were weak or anemic before the treatment, but there is no particular alteration in the mortality.

It is fair to note that the action of a given substance is not always the same toward different tumors and in different species. Therefore, if the claims of partial tumor changes can be verified with nonfatal doses of lead in clinical cases, there may still be a possibility of its use as an adjuvant to other methods of treatment.

OTHER COLLOIDS

On account of the statements of authors, such as Nenberg and Caspari,³ concerning the tumor-killing action of various metals, and also the emphasis placed by other writers upon the special advantages of the colloid state, much time and many animals were devoted to an investigation of intravenously injected substances other than lead. Particular attention was paid to colloidal arsenic, partly because of the reports of Hendrick and Burton.¹² The other colloidal solutions included gold,¹³ silver, iron,^{14, 15} manganese, copper, antimony, sulfur, and selenium.¹⁶ Attempts were made by combinations and alternations of these to obtain a cumulative action upon the tumors, at the same time avoiding the toxicity of the individual elements, but failure resulted because the general toxic action was cumulative. In particular, it was hoped that an advantageous combination with ligation might be found by injecting immediately before ligating so that the circulating material would be held in the tumor for several hours, or by injecting immediately after release of the tourniquet so that the reactive hyperemia would flood the tumor with a maximum amount of the material. In either instance it was hoped that the asphyxiated tumor might be sensitized to the poison. All results were negative, without a single cure. Such cures as might have been expected from ligation alone were prevented by the fact that the intoxicated animals could not survive the usual duration of ligation.

DYES

Dyes have been among the most favored chemotherapeutic agents in attempts to influence cell respiration or anchor toxic substances to the tumor.¹⁴ It has been customary to search for semidiffusible substances, which would pass from the blood into the tumor and remain there for a sufficient period of time. The special conditions of ligation, already mentioned, place a premium upon the freest possible diffusibility and provide favorable opportunities for any selective action.

No experiments were performed with systemically injected dyes. A few trials were made with single or repeated local injections of dilute aqueous solutions of eosin, methylene blue,¹⁷ or gentian violet, during ligation of different rat tumors, mouse sarcoma 180, and chicken sarcoma. No cures and no improvement in results were obtained. Irregularities in distribution of the dyes were plainly visible. Addition of eosin or methylene blue to the usual arsenic solutions gave no improvement of results as compared with arsenic alone. These dyes may have been poorly chosen for diffusibility or other qualities, and the results may perhaps be improved under better technical conditions.

OTHER AGENTS AFFECTING RESPIRATION

Attempts to attack the tumor cell through its respiration have been too numerous to review.^{14, 18} The earliest stage of this research in the Physiatrie Institute included keeping the animals in glass chambers under various reduc-

tions or increases of oxygen pressure. Later some trials were made with chemical agents, such as potassium cyanide, to depress respiration, and with dinitrophenol to increase cell metabolism.¹⁹ Oxygen enrichment was also undertaken by subcutaneous and intraperitoneal injections of oxygen several times in each of the twenty-four hours, which sufficed to maintain an abnormally bright color of the venous blood. Chronic carbon monoxide poisoning was established by this same injection method, using ordinary illuminating gas. Preliminary tests determined the minimum lethal doses of these injections for normal animals, which could thus be kept saturated to such a point that the blood remained cherry red and death resulted from a slight increase of the injections. The only new principle involved was the systemic injection of the above agents in connection with local ligation, as well as local injections of the solutions and gases directly into the region of the ligated tumor. Although these experiments in the aggregate represented much work, the results were so completely negative as to require no detailed description.

STREPTOCOCCUS TOXIN

A few intravenous injections of Coley fluid²⁰ were tried, together with a larger number of local infiltrations with this fluid, or living or killed streptococcus cultures. Special attention was given to repeated infiltrations, and particularly to various combinations with ligation. The tissue reaction in the rat is different from that in man, so that the indurated swellings which are familiar clinically¹⁸ are not obtainable in the rat. Neither a curative tendency nor the slightest indication of a selective toxicity of the streptococcus products for tumor as compared with normal tissues was found.

Streptococcus toxin can best be classified along with arsenic in its action upon the blood vessels. The principal error in dealing with such remedies has consisted in the search for a specific toxic action on tumor cells. The correct approach must place emphasis upon the vascular and tissue reactions, which vary with animal species, tumors, and locations of the tumor. Negative results in rats, therefore, by no means exclude the usefulness of either arsenic or streptococcal toxin under properly chosen clinical conditions, as will be shown in a subsequent publication.¹⁸

CONCLUSIONS

1. The action of certain drugs, especially arsenic, and to a less extent lead, strikingly resembles that of local asphyxia and the mechanism of the tumor-killing effect may be similar. The attempts to use local ligations to obtain an intensified action of the drugs, or to use the drugs in local or systemic application to augment the effect of asphyxia, on the whole gave scanty success because of insufficient specificity, the distinctly increased injury to the tumors being offset by increased local or systemic dangers.

2. In conformity with previous work indicating that the tumor-killing action of local ligation is due neither to the direct asphyxia nor to the excess oxygenation of the ensuing hyperemia, the combination of ligation with various agents serving to increase or to depress cell respiration proved negative.

3. Notwithstanding the generally inconclusive results, some emphasis should be placed on the fact that cures with arsenic were obtained in some mouse

tumors and also in favorably situated rat tumors. There are undoubtedly differences in efficacy, depending upon the location and the specific sensitiveness of different tumors, and an application of this experience for an improved arsenic therapy of certain clinical tumors will be described elsewhere.¹⁴

REFERENCES

1. Allen, F. M.: Local Asphyxia and Temperature Changes in Relation to Gangrene and Other Surgical Problems, *Tr. A. Am. Physicians* 52: 189, 1937.
- Ligation and Refrigeration of Intestine, *Surgery* 3: 893, 1938.
- The Tourniquet and Local Asphyxia, *Am. J. Surg.* 41: 192, 1938.
- Resistance of Periplural Tissues to Asphyxia at Various Temperatures, *Surg. Gynec. & Obst.* 67: 746, 1938.
- Effects of Ligations on Nerves of the Extremities, *Ann. Surg.* 108: 1088, 1938.
- Physical and Toxic Factors in Shock, *Arch. Surg.* 38: 155, 1939.
- Surgical Considerations of Temperature in Ligated Limbs, *Am. J. Surg.* 45: 459, 1939.
- Experiments Concerning Ligation and Refrigeration in Relation to Local Intoxication and Infection, *Surg. Gynec. & Obst.* 68: 1047, 1939.
- Mechanical Treatment of Venomous Bites and Wounds, *South. M. J.* 31: 1248, 1938.
- Observations on Local Measures in the Treatment of Snake Bite, *Am. J. Trop. Med.* 19: 393, 1939.
- Transfusions and Polycythemia in Normal and Tumor-bearing Rats, *J. Lab. & Clin. Med.* 25: 471, 1940.
- Influences of Local Asphyxia, Organ Extracts and Temperature Changes on Engrafted Subcutaneous and Visceral Tumors in Rats, *Urol. & Cutan. Rev.* 44: 1, 1940.
- Local Asphyxia and Tumors, *Am. J. Surg.* 49: 484, 1940.
- Ligation Treatment of Chicken Sarcoma, *Med. Rec.* 152: 87, 1940.
- Action of Ergot in Normal and Tumor-Bearing Rats, *Med. Rec.* 152: 351, 1940.
- Possible Factors in the Reaction of Tumors to Local Asphyxia, *Am. J. Clin. Path.* 10: 813, 1940.
- 1a. Allen, F. M.: Effects of Local Reaction in Spontaneous Tumors of Animals and Man, *Arch. Surg.* 41: 79, 1940.
2. Allen, F. M.: Local Asphyxia and Roentgen Irradiation of Transplantable Rat Tumors, *Am. J. Roentgenol.* 42: 745, 1939.
3. Neuberg, C., Caspari, W., and Löhle, H.: *Weiters über Heilversuche an geschulstkranken Tieren mittels tumoraffinen Substanzen*, *Berl. klin. Wchnschr.* 49: 1405, 1912.
4. Woglom, W. H.: *The Study of Experimental Cancer*, New York, 1913, Columbia University Press.
5. Fischer, A.: *Embryonale Zellen und Krebszellen*, *Klin. Wchnschr.* 6: 433, 1927.
- White, A. W. M.: A Study of Sarcoma of the Fowl Produced by Arsenic and Embryonic Pulp, *J. Cancer Research* 11: 111, 1927.
- McNeer, G.: Arsenical Keratoses and Epitheliomas, *Ann. Surg.* 99: 348, 1934.
6. Hueper, W. C., and Itami, S.: Effects of Neoparsphenamine on Spontaneous Breast Tumors of Mice, *Am. J. Cancer* 17: 106, 1933.
7. Cuslly, A. R.: *Pharmacology and Therapeutics*, ed. 9, Philadelphia, 1928, Lea & Febiger.
8. Solimann, T.: *Manual of Pharmacology*, ed. 5, Philadelphia, 1936, W. B. Saunders Co.
9. Willberg, M.: Zur Frage nach der Resistenz verschiedenen Tiere gegenüber arsen, *Biochem. Ztschr.* 51: 231, 1913.
10. Blair-Bell, W.: The Influence of Saturnine Compounds on Cell-growth, With Special Reference to the Treatment of Malignant Neoplasms, *Lancet* 2: 1005, 1922.
- Symposium on the Nature of Malignant Neoplasia and Treatment of the Disease With Lead, *Brit. M. J.* 2: 919, 1926.
- Some Aspects of the Cancer Problem, London, 1930, Balliere, Tindall & Cox.
- Weller, C. V.: The Blastophoric Effect of Chronic Lead Poisoning, *J. M. Research* 33: 271, 1915.
- Stone, W. S., and Craver, L. F.: A Clinical Study of the Colloidal Treatment of Malignant Neoplasms, *J. Cancer Research* 11: 204, 1927.
- Lewis, W. C. M.: Some Physico-chemical and Biochemical Aspects of Malignant Neoplasms, *Brit. M. J.* 2: 920, 1926.
- Aub, J. C., and Smithwick, R. H.: Lead Treatment of Cancer, *New England J. Med.* 208: 310, 1933.
11. Kraemer, W. H.: Cancer Problem, Present and Future, *Am. J. Roentgenol.* 25: 793, 1931.
- Preliminary Report on Colloidal Lead Phosphate With Manganese, *Am. J. Cancer* 15: 2357, 1931.
12. Hendrick, A. C., and Burton, E. F.: Changes in Bone Tumours After Intravenous Injections of a Colloidal Solution, *Canad. M. A. J.* 24: 642, 1931.
- Changes in Bone Sarcoma After Intravenous Injection of a Colloidal Solution of Metallic Arsenic, *Canad. M. A. J.* 28: 192, 1933.

13. Vallery-Radot, P., Gilbrin, E., and Ganthier-Villars, P.: Néphrites expérimentales par les sals d'or. Parallélisme entre l'élimination du métal et des lésions histologiques, *Ann. de méd.* 37: 145, 1935.
14. Fischer-Wasels, B.: Die Behandlung bösartiger Geschwülste mit Sauerstoff-Kohlensäuregemischen in Verbindung mit Eisen und Eisenfarbstoffen, Frankfurt. *Ztschr. f. Path.* 39: 1, 1930.
Experimentelle Grundlagen und folgerungen der Regenerationstheorie der Geschwülstbildung, *Klin. Wehnschr.* 11: 1937-1941, 1977-1982, 1932.
Die Entwicklung der Geschwülstzelle, *Klin. Wehnschr.* 6: 1025-1029, 1073-1077, 1927.
Die Bedeutung der besonderen " " " " des Körpers für die Entstehung der Krebskrankheit und die " " " " Bekämpfung, *Strahlentherapie* 50: 5, 1934.
- Magot: Versuche mit Phosphatid-Peroxyden bei Tumormäusen, ratten, kaninehen (I Mitteilug), *Ztschr. f. Krebsforsch.* 39: 219, 1933.
15. Romhányi, J., and Schmidt, M.: Ueber den Eisenhaushalt krebskranker Tiere, Frankfurt. *Ztschr. f. Path.* 48: 380, 1935.
- Sell, H., and Carls, H.: Der Stoffwechsel der Careinomzelle unter dem Einfluss oxydationsfördernder und oxydationshemmender Faktoren, *Ztschr. f. Krebsforsch.* 40: 416, 1934.
- Warburg, O.: Ueber Eisen, den Sauerstoff-übertragenden Bestandteil des Atmungsfermentes, *Ber. d. dtseh. chem. Gesellsch.* 58: 1001, 1925.
16. Todd, A. T.: The Selenide Treatment of Cancer, *Brit. J. Surg.* 21: 619, 1934.
17. Warburg, O.: Mechanismus der Methylenblauatmung, *Ann. Surg.* 93: 205, 1931.
- Brooks, Matilda M.: Effect of Methylene Blue on Tumors, *Proc. Soc. Exper. Biol. & Med.* 30: 1001, 1933.
18. Fischer-Wasels, B.: Ueber Gasbehandlung bösartiger Geschwülste und kachektischer Zustände, *Klin. Wehnschr.* 7: 53-57, 106-110, 153-159, 1928.
- Campbell, J. A.: The Use of Gas Mixtures in Cancer Therapy, *Cancer Rev.* 6: 230, 1931.
19. Muntwyler, E.: Effect of 1-2-4-dinitrophenol on Oxygen Uptake of Rat Tissue, *Proc. Soc. Exper. Biol. & Med.* 32: 1060, 1935.
- Krahl, M. E., and Clowes, G. H. A.: Observations on Cellular Oxidative Mechanisms Involved in Dinitrophenol Stimulation of Respiration, *Proc. Soc. Exper. Biol. & Med.* 32: 226, 1934.
- Terada, B. J., and Tainter, M. L.: Metabolic Response of White Rats to Continued Administration of Dinitrophenol, *J. Pharmacol. & Exper. Therap.* 54: 454, 1935.
- DeMeio, R. H., and Barron, E. S. G.: Effect of 1-2-4-dinitrophenol on Cellular Respiration, *Proc. Soc. Exper. Biol. & Med.* 32: 36, 1934.
- McCord, W. M.: The Effect of 2-4 dinitrophenol on the Oxygen Uptake of Kidney and Liver Tissue of the Rat, *Am. J. Physiol.* 109: 232, 1934.
20. Coley, W. B.: Late Results of the Treatment of Inoperable Sarcoma by the Mixed Toxins of Erysipelas and Bacillus prodigiosus, *Am. J. M. Sc.* 131: 375, 1906.

EXPERIMENTAL UTEROTUBAL INSUFFLATION IN THE RABBIT*

A PRELIMINARY REPORT

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FALLOPIAN tube physiology has been investigated since 1925 by means of transuterine insufflation of gas according to the method of Rubin.¹ Essentially, this method consists of recording the fluctuations in pressure of gas insufflated through the uterus and tubes at a relatively constant rate. Feresten and Wimpfheimer² have demonstrated the similarity in the response of the rabbit and man to gas insufflation. In studying this field, one is immediately confronted with certain basic problems. How is the pressure curve affected by the type of gas employed, simple repetition of insufflation, rate of gas flow, intra-abdominal pressure, anesthesia, asphyxia, and number of functioning Fallopian tubes connected with the uterus? We have sought preliminary data to answer these questions.

METHOD

Virgin female rabbits, weighing 1 to 2 kg., were used. When anesthetized with nembutal, one grain per kilogram was injected intravenously, providing thorough anesthesia for thirty minutes, and, when the animals awoke, they remained quiet several hours for further investigation. The body temperature did not fall significantly throughout the experiments and no gross changes in cardiac and respiratory rates were observed unless the abdomen was permitted to distend too rapidly with gas.

Most of the experiments were performed by the technique of Feresten and Wimpfheimer.² A hypogastric incision was made and the body of one uterus was delivered into the wound. Through a small incision into the uterine wall, about 1 cm. proximal to the cervix, a cannula was directed cephalad and fixed by a ligature which encircled the entire circumference of the uterus to prevent gas from regurgitating through the cervix. The uterus and distal end of the cannula were replaced into the abdomen; the wound was temporarily closed with Allis clamps, and a small opening was made into the epigastrium to permit the free escape of gas.

Some studies were performed on animals with uterine fistulas made according to the technique of Reynolds.³ Under sterile precautions the vagina was drawn through a small suprapubic incision and divided. The distal portion of the vagina was ligated with silk to prevent peritoneal contamination with urine. The proximal end was drawn through a small stab wound in the midline, cuffed back and sutured to the skin to exteriorize the two cervices. These animals were insufflated without the use of anesthesia, using a clinical

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type of cannula with a rubber acorn to prevent the regurgitation of gas. One animal was insufflated daily for two weeks and post-mortem examination failed to reveal any evidence of uterine or pelvic infection.

The cannulae were connected to the usual clinical Grafix Kymographic Insufflation apparatus. This machine employs an anaeroid recording manometer which requires 48 c.c. of gas to record a pressure range of 200 mm. Hg. The clinically available mercurial float type of manometer was found less sensitive, requiring 62 c.c. for a similar pressure range. The insufflating gases were carbon dioxide and oxygen supplied from tanks at 15 pounds pressure. A valve permitted controlled variation in the rate of gas flow. Of necessity, the gas rate cannot be maintained truly constant because the flow rate during insufflation varied according to the pressure differential between the tank source of 760 mm. Hg and the recording kymographic range from 0 to 200 mm. Hg. Constant rates of flow, with pressure measured by an optical manometer, were obtained with fluid rather than with gas by means of a motor-driven syringe. The pressure changes with this medium did not resemble the usual gas insufflation curves and we are at present investigating the mechanism of this discrepancy.

To study the effect of death on insufflation, 30 c.c. of air were injected intravenously. The animal's respirations ceased immediately, and after several convulsive movements the animal became immobile. The heartbeat ceased several minutes later.

EXPERIMENTAL FINDINGS

In the analysis of a typical kymographic tracing, three features were studied: (1) The *primary pressure*, which is attained before gas enters the abdominal cavity, was manifest on the tracing by a characteristic maxima high peak followed by a precipitous fall until a plateau level is reached which is (2) the *general level of pressure*; this is maintained throughout an insufflation and about which (3) *small waves of pressure* fluctuate. These may be superimposed upon larger waves.

Effect of Different Insufflating Gases.—In this investigation the two biologically most important gases, carbon dioxide and oxygen, have been studied in most of the following experiments. Frequent differences have been observed between these gases. The effect of such inert gases as helium and nitrogen and the anesthetic gases are now under investigation.

Effect of Variable Rates of Gas Flow.—In an animal as small as a rabbit with a Fallopian tube luminal diameter of approximately 0.75 mm., small variations in the rate of flow of gas produced changes in the curves. A increase in the rate of flow generally raised the primary pressure level, the general level of insufflating pressure, the size and to a less extent the frequency of the small waves of pressure. At the very slow rates of gas flow some of the small waves of pressure may be submerged due to the low sensitivity of the manometer. Unless indicated, all further experiments were conducted with as constant a rate of flow as could possibly be maintained during a single experiment.

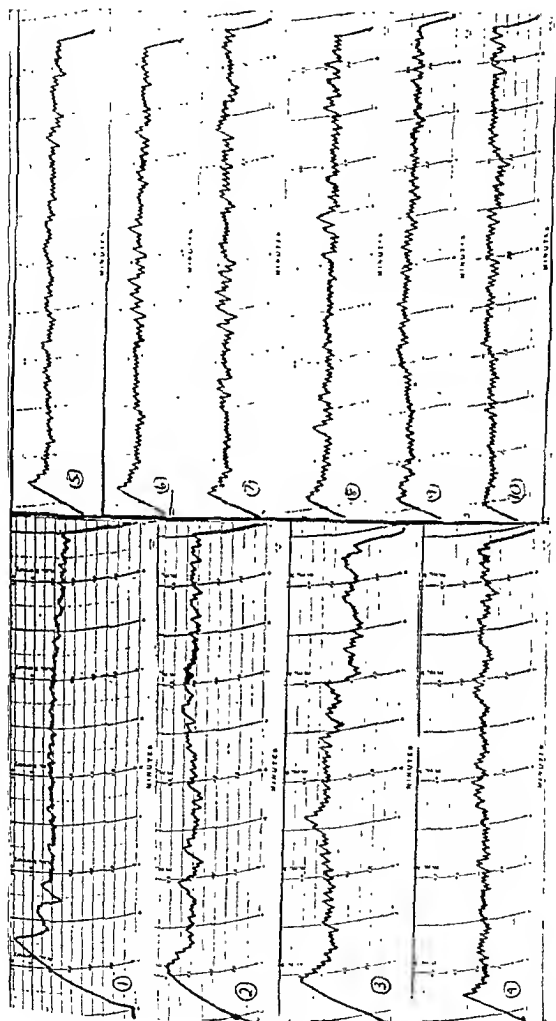


FIG 1.—Ten consecutive insufflations with oxygen

Effect of Repeated Consecutive Insufflations.—These animals were insufflated ten times consecutively with each insufflation lasting ten minutes and a rest period of twenty to forty-five seconds between tracings. Of twenty animals, oxygen was used in ten and carbon dioxide in the other ten; slightly different results were obtained.

1. The primary pressure at which gas enters the abdominal cavity generally becomes less on repeated insufflations, reaching the general level of insufflation pressure after about seventy minutes of insufflation. In one animal no gas entered the abdominal cavity on the first insufflation, although a pressure of 200 mm. Hg was reached; on subsequent insufflations the gas entered at progressively lower levels.

2. The general level of pressure also fell on repeated insufflations and remained comparatively stable after seventy minutes.

3. The frequency of the small waves usually decreased if carbon dioxide was used, although in a rare tracing the rate may return to that of the first insufflation. When oxygen was used, the frequency tended to remain more constant (Figs. 1, 2). With both gases the amplitude of the small waves tended to increase (Fig. 2).

4. In one animal, following insufflation of the right uterus for fifty minutes, the left uterus was insufflated. The tracing of the left uterus resembled more that of the first tracing of the right uterus than its fifth (Fig. 3).

Effect of Anesthesia.—Animals were insufflated without anesthesia or under procaine infiltration of the anterior abdominal wall and then reinsufflated after inducing anesthesia to compare the character of the tracings.

1. Nembutal produced no discernible effects in the dosage employed.

2. Ether anesthesia produced diverse effects depending on the gas employed. With carbon dioxide there was a gradual lowering of the general level of pressure and a diminution in the frequency and amplitude of waves (Fig. 4). Often under deep ether narcosis, the manometer recorded zero with no waves. When oxygen was used, the tracing remained normal with the exception of a marked rise in the general level of pressure, even though the animal may be anesthetized to the point of death. This suggests that anesthesia might play a role in the clinical diagnosis of apparent "closed tubes."

Effect of Increased Intra-abdominal Pressure.—Moderate and even marked abdominal distention, if produced gradually (as by obturating the vent in the upper abdomen and permitting the insufflated gas to remain in the abdominal cavity), had no effect upon the curves. The changes produced in intra-abdominal pressure by quiet respiration produced no observable changes in the tracings. However, a sudden increase in intra-abdominal pressure always caused the small waves to fluctuate about a higher general level of pressure. Exciting the unanesthetized animal by pinch stimulation of the ear caused a sudden rise in intra-abdominal pressure and a rise in the general level of pressure recorded on the kymograph. When the abdomen was opened widely to expose the cavity to free atmospheric pressure, repeated stimulation of the ear caused no change in the tracing (Fig. 5).

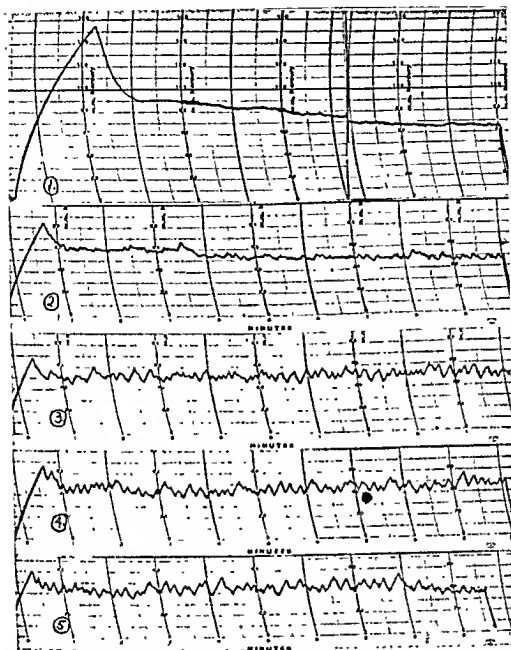


Fig. 2.—Five consecutive insufflations with oxygen, demonstrating the progressive rise in height of the waves.

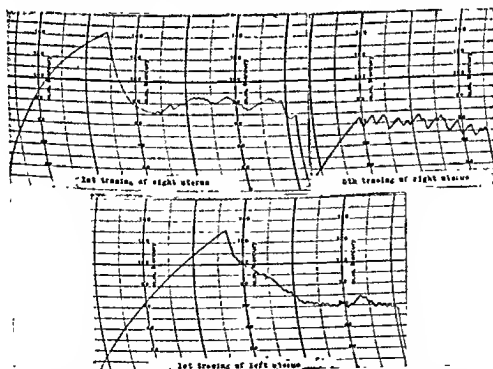


Fig. 3.—Comparison between the first tracing of the left uterus and the first and fifth consecutive tracings of the right uterus.

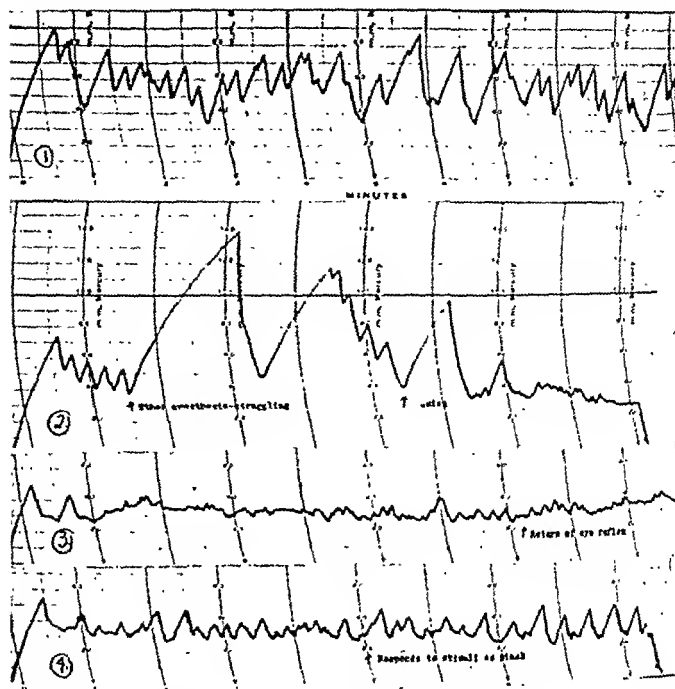


Fig. 4.—Effect of ether anesthesia on an animal insufflated with carbon dioxide with control period of rest.

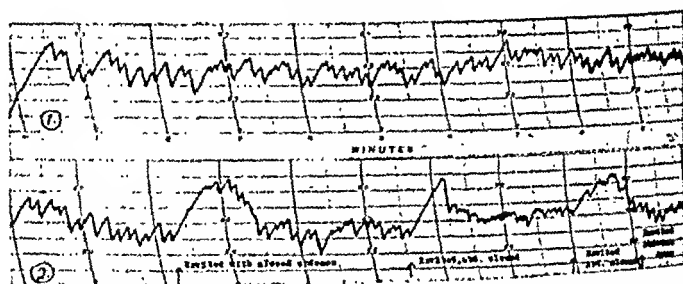


Fig. 5.—Effect of excitation with closed and open abdomen in an animal insufflated with carbon dioxide.

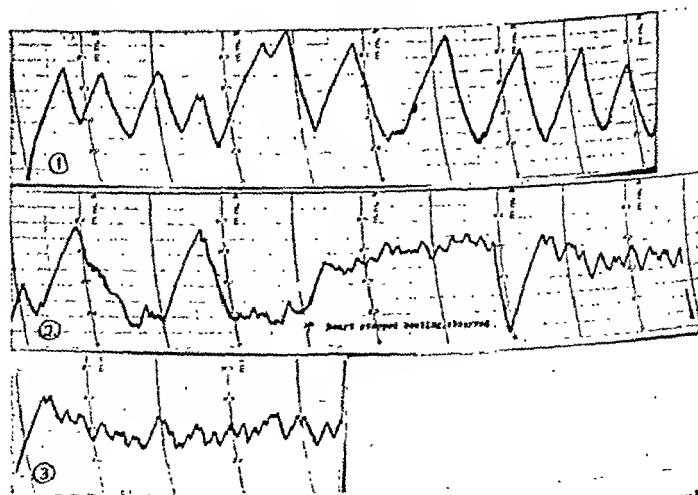


Fig. 6.—Insufflation with oxygen in an animal asphyxiated with excessive nembutal and the change in the tracing upon death of the animal.

Effect of Asphyxia.—In the case of two animals insufflated with carbon dioxide, the dosage of nembutal was apparently excessive, and asphyxia, evidenced by cyanosis and infrequent, shallow respirations, resulted. In these cases very deep and slow undulating waves replaced the usual quick shallow waves of pressure.

An asphyxiated animal, insufflated with oxygen, presented a similar type of curve, but as soon as its heart stopped beating, the tracing returned to a normal variety (Fig. 6). This suggests that an excessive blood carbon dioxide may change the character of the waves.

Insufflation of Dying and Dead Animals.—As soon as respirations of the animals ceased, even though the abdomen was kept open to obviate the effect of increased abdominal pressure induced by the convulsive movements, the general level of insufflating pressure rose to huge heights. When carbon dioxide was used, the curve gradually fell to low levels, with irregular small waves and, on cessation of the heartbeat, the pressure fell to zero and all small waves disappeared (Fig. 7).

When oxygen was used, the characteristic agonal high pressure level was observed and then the pressure fell gradually to a normal level and a normal curve resulted and was maintained for more than twenty-five minutes, although in one animal, the heart had been removed and its lumbar spine transected to produce practically an *in vitro* preparation.

Insufflation with either gas of animals that had been dead for a long time failed to elicit any contractions or any rise in the tracing unless the gas was passed at very rapid rates of flow, when the resultant tracing merely represented the pressure relations produced by the passage of gas through narrow, inanimate channels.

Effect of Simultaneous Insufflation of Both Uteri.—The insufflation of the human uterus with its single cavity and two tubes or the simultaneous insufflation of both uteri in a rabbit suggests two theoretical dynamic possibilities:

1. The resultant tracing is actually produced by gas passing through only one tube.
2. The gas passes through both tubes. This latter possibility brings up the question as to whether the two tubes contract and relax synchronously.

In each of two rabbits both uteri were cannulated and connected by a Y tube to the insufflation apparatus. By appropriate clamps on the tubing each uterus was insufflated in turn and finally both simultaneously. During the bilateral insufflation the gas was observed to bubble from only one tube, the one whose primary pressure level of gas entrance was the lower (Fig. 8).

Kiag⁴ with the aid of a double stethoscope reported that in insufflation of human beings, the gas passed either through one or the other tube or through both at once. The pneumoperitoneum produced by insufflation in man has been frequently unilateral. If the gas were to pass through both tubes, the resultant curves in man should exhibit greater variation in the amplitude and frequency of the small waves, with increasing asynchrony of the tubes. Further investigations along this direction are being performed in man.

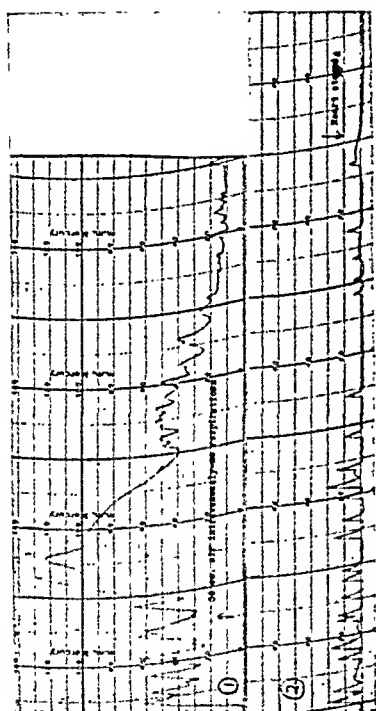


Fig. 7.—Effect of intravenous air upon an animal insulated with carbon dioxide.

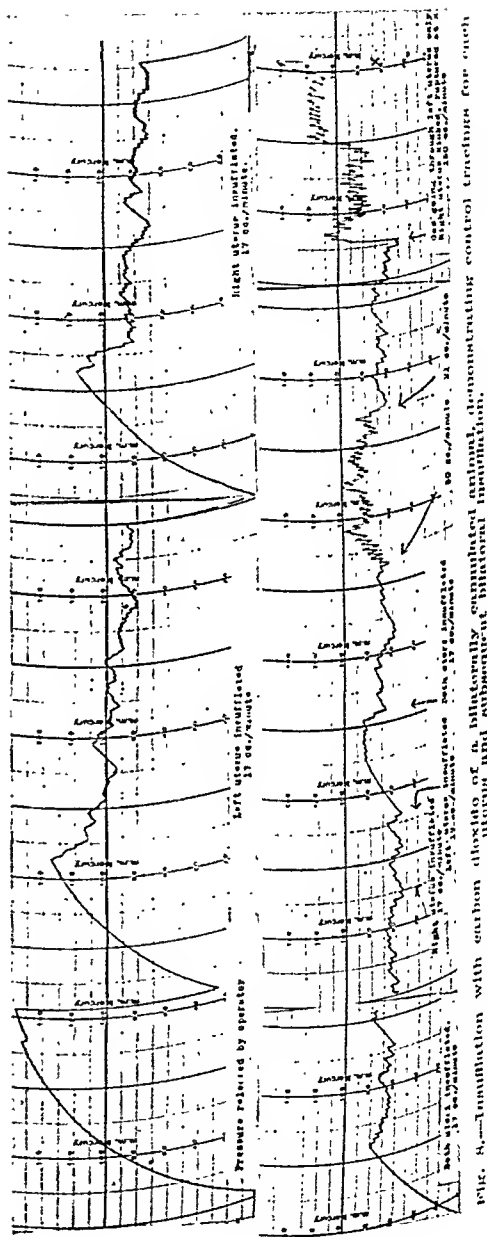


Fig. 8.—Insulation with carbon dioxide of a bilaterally extirpated animal, demonstrating control tracings for such a case and subsequent bilateral insulation.

CONCLUSIONS

1. The type of the curve varies slightly with the kind of insufflating gas.
2. The flow of gas must be kept constant and adequate for the sensitivity of the manometer.
3. Inasmuch as repetition of insufflation modifies the character of the curve, definite criteria must be established to evaluate the curves obtained under experimental conditions as in the investigation of drug and hormonal action.
4. Various anesthetics exert different effects upon the kymographic curves.

I wish to acknowledge the kind advice of Dr. Max Mayer and Dr. Arthur Ginzler and the technical assistance of Mrs. Elizabeth Dreifus.

REFERENCES

1. Rubin, I. C.: Diagnostic Value and Therapeutic Application of Peruterine Insufflation of Fallopian Tubes in Cases of Sterility, *Proc. Roy. Soc. Med.* 19: 1, 1925.
2. Feresten, M., and Wimpfleimer, S.: Patency of the Uterotubal Junction of the Rabbit. Experimental Observations With the Aid of CO₂ Insufflation (Rubin Method), *Endocrinology* 24: 510, 1939.
3. Reynolds, S.: Studies on the Uterus; Method of Recording Uterine Activity in Chronic Experiments on Unanesthetized Animals, *Am. J. Physiol.* 92: 420, 1930.
4. King, G.: Double Stethoscope; Aid in Determining Status of Individual Tubes During Performance of Rubin's Test, *Surg., Gynec. & Obst.* 63: 76, 1936.

TREATMENT OF POLYCYTHEMIA VERA WITH LIVER AND CHOLINE HYDROCHLORIDE*

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DAVIS¹ has recently reported that polycythemia induced in dogs by exercise on a treadmill, exposure to reduced pressure in a chamber, or by the administration of cobalt was modified significantly with reduction in the numbers of erythrocytes when whole raw liver or liver extract was administered. In the dogs made polycythemic by cobalt administration or reduced pressure the oral administration of choline hydrochloride, 8 mg. per kilogram in 1 per cent solution per day, also caused diminution in the erythrocyte count after two or three doses.

The mechanism of the production of polycythemia with cobalt is not established with certainty, but it is probably a result of bone marrow stimulus without a physiologic need for this stimulus. The polycythemia of exercise is, of course, but relative, whereas that of reduced pressure is compensatory for a diminished oxygen tension. Therefore, it seemed singular that the same type of therapy could cause reduction in the number of erythrocytes in all three types. Nevertheless, in view of the reported results and the suggestion of Davis that choline may be of benefit in treating polycythemia vera, 5 patients, none of whom had received any previous treatment of any kind for their primary disease, were given either whole raw liver, choline hydrochloride, or both. Raw liver is a good source of choline, containing not less than 1 per cent.²

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TABLE I
COMPARISON OF BLOOD FINDINGS BEFORE AND AFTER LIVER AND CHOLINE ADMINISTRATION IN POLYCYTHEMIA VERA

	PATIENT 1 (211,283)		PATIENT 2 (110,726)		PATIENT 3 (211,651)		PATIENT 4 (94,222)		PATIENT 5 (211,985)	
	10/9/39	10/19/39	10/18/39	11/7/39	10/30/39	11/15/39	10/30/39	12/2/39	11/21/39	12/7/39
Hemoglobin (%)	138	130	135	141	168	149	134	125	141	157
Erythrocytes	6,175,000	6,245,000	6,258,000	6,595,000	8,388,000	7,263,000	8,945,000	8,250,000	6,478,000	6,838,000
Reticulocytes (%)	0.8	0.9	0.3	1.0	1.0	0.9	2.1	3.0	0.7	1.0
Hematocrit	60.0	56.7	63.2	66.5	80.7	68.7	66.5	65.4	69.8	67.6
Plasma, blood volume (c.c.)	2,784		2,300				3,912	4,542	2,970	
Total blood volume (c.c.)	7,579		6,084				13,321	12,784	9349	
Patient's weight (kg.)	63.5		56.3		71.0		72.6	65.4	68.2	
	Received 300 Gm. of raw ground liver daily for 10 days		Received 300 Gm. of raw ground liver daily for 19 days		Received 300 Gm. of raw ground liver daily for 14 days		Received 300 Gm. of raw liver daily for 32 days		Received 600 mg. of choline hydrochloride daily for 9 days after initial dose of 300 mg.	
									Received 550 mg. of choline hydrochloride daily for 12 days after initial dose of 250 mg.	

METHODS

All patients had complete blood counts done; the erythrocyte counts in duplicate, hemoglobin determinations using the Haden-Hausser hemoglobinometer (15.4 Gm. per 100 c.c. equaling 100 per cent); reticulocyte counts on stained smears, the percentage being derived from examining 1,000 erythrocytes; hematocrit determinations using Wintrobe tubes, and blood volume determinations (in four of the five patients as further diagnostic data by the method described by Gibson and Evelyn³). The total and differential leucocyte counts are not reported since there were no significant alterations during treatment in any case.

In patient 2, the only one followed as an outpatient, blood studies were done previous to therapy and again at its completion. In the other four patients complete blood counts, reticulocyte counts, and hematocrit determinations were done twice each week throughout each period of observation. For brevity's sake, however, only the data acquired before therapy and at the completion of each period of study are given.

Each of the four patients treated with liver received 100 Gm. of fresh ground raw calf's liver with salt, pepper, and catsup added, or in fruit juice, three times a day. The patients who received choline hydrochloride got 8 mg. per kilogram body weight in 1 per cent solution. The five patients were males and their respective ages were 64, 51, 54, 20, and 59. They all took choline hydrochloride and liver with little or no difficulty.

DISCUSSION

The results appear to be clearly negative in four patients as far as reduction of erythrocytes or hemoglobin is concerned, for there was no significant change in any patient while undergoing liver or choline therapy. In patient 3, who received liver therapy, there was a decrease in hemoglobin and erythrocytes, and in the hematocrit reading. This diminution had its inception on the tenth day of treatment and was of the same degree on the fourteenth day. During the period of observation, before a fall in the blood levels was demonstrable, a wet granular ulcerative lesion on the leg was present. Coincident with changes in the blood, complete healing occurred. Whether hemoconcentration may have played a part in explanation of the previous very high hemoglobin and erythrocyte levels is problematical. Attempts at determining the blood volume preceding therapy were unsuccessful since pronounced viscosity of the blood and prompt coagulation interfered with collection of blood samples.

Marshall⁴ has reported that cobalt polycythemia in rats is modified with reduction in the erythrocyte count following liver extract or ventriculin administration but not after feeding fresh whole liver. Stephan⁵ has reported that whole fresh liver was of clinical value in treating the polycythemia symptomatic of a variety of conditions, but Major⁶ noted no benefit from liver extract administration in three cases of polycythemia vera. We have treated no case of polycythemia with liver extract. Ogawa⁷ successfully treated one case of polycythemia vera with oral and parenteral liver substance, but the polycythemia recurred with the cessation of therapy.

Our results with liver therapy in polycythemia vera are not absolutely conclusive for during therapy one patient showed a decrease in erythrocytes and

hemoglobin, although there was an extenuating circumstance in this case. In the other two patients treated with raw calf's liver alone, in the one patient treated with liver and later with choline hydrochloride, and in the one patient treated with choline alone there were no significant changes in the blood levels. It is our interpretation that the administration of raw calf's liver or choline hydrochloride is probably without value in the treatment of polycythemia vera.

We wish to acknowledge the kind cooperation of Dr. Marian S. Kimble who did the blood volume determinations.

REFERENCES

1. Davis, J. E.: Reduction of Experimental Polycythemias by Liver Administration, *Am. J. Physiol.* 122: 397, 1938; Depression of Experimental Polycythemias by Choline Hydrochloride or Liver Administration, *Ibid.* 127: 322, 1939; Depression of Polycythemia by Choline Hydrochloride, *Proc. Soc. Exper. Biol. & Med.* 40: 445, 1939.
2. Jacobs, H. R.: On Nature of Antipernicious Anemia Principle, Search for Nitrogenous Bases Isolation of Choline, *J. LAB. & CLIN. MED.* 24: 128, 1938.
3. Gibson, J. G., and Evelyn K. A.: Clinical Studies of Blood Volume; Adaption of Method to Photoelectric Microcolorimeter, *J. Clin. Investigation* 153: 17, 1938.
4. Marshall, L. H.: Antianemic Treatment in Experimental Polycythemia, *Am. J. Physiol.* 114: 194, 1935.
5. Stephan, R.: Symptomatische Polyglobulie und Lebersubstanz-Therapie, Ein Beitrag zur Pathogenese der perniciösen Anämie, *Klin. Wchnschr.* 9: 1068, 1930.
6. Major, R. H.: Effect of Liver Extract Upon Polycythemia Vera, *J. LAB. & CLIN. MED.* 24: 65, 1938.
7. Ogawa, J.: Über den Einfluss der Lebersubstanz auf Polycythemia vera, zugleich ein Beitrag zur Milzdättherapie, *Jap. J. M. Sc.* 2: 267, 1932.

FURTHER STUDIES ON THE EFFECT OF MASSIVE INTRAVENOUS INFUSIONS IN NARCOTIC POISONING*

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IN A PREVIOUS communication¹ we described the effects of massive intravenous infusions of isotonic glucose or saline solutions, in volumes varying from one-fourth to three-fourths the body weight, on the duration of barbiturate narcosis. It was found that infusions of such volumes of fluid accelerated the time of recovery of anesthetized animals and prevented death in those receiving fatal or several fatal doses of these drugs. It was suggested that the method of treatment with massive intravenous infusions may similarly prove antidotal to all slowly acting neurotoxic drugs, particularly central depressants. The present report deals with the experimental exploration of this hypothesis.

Dogs were used in all the experiments. For the technique of administering massive intravenous infusions reference should be made to the preceding paper.¹ A solution of 1 per cent sodium chloride was used for infusions in this series. It should be emphasized in this connection that care in the technique of the preparation of solutions to be infused in quantities such as we have used

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is essential. This is to avoid the possibility of contamination with pyrogenic agents and other harmful contaminants possibly present in water. Scrupulous asepsis should be observed throughout, and the rates of infusion should be carefully controlled. One of the principal mechanisms likely to cause fatalities from massive infusions is insufficient diuresis. It is the retained fluid, rather than the total amount infused, which is critical. Animals withstand more rapid rates of infusion and larger total quantities of solution when the kidneys eliminate urine in considerable quantities. The effect of massive infusions was tested on the course of poisoning by oral, hypodermic, or intravenous fatal doses of the following substances: methyl alcohol, ethyl alcohol, chloral hydrate, sodium bromide, and potassium arsenite. Whenever oral doses were given, the animals also received a preliminary injection of 10 mg. per kilogram of body weight of morphine sulfate* intramuscularly to abolish the vomiting reflex and thus insure retention of the dose. Inasmuch as morphine is a well-known synergist to all aliphatic narcotics, one may assume that morphine enhanced the toxicity of the orally administered drugs.

Many animals not listed in the following discussion and tabular protocols were used during the course of the experiments. For every dose of methanol, ethanol, chloral hydrate, and sodium bromide here reported, doses of similar magnitude were administered to two or more animals which died before infusion could be instituted, and others receiving such doses died during the infusions. All these may be regarded as constituting control experiments and as supporting the observations of other investigators that all doses used were considerably in excess of the ordinary fatal dose.

Methanol.—Methanol was administered by mouth in two animals thirty minutes following a preliminary injection of morphine to control vomiting. Absolute methanol in doses of 20 c.c. diluted with water was given to each animal. No regurgitation or vomiting occurred. The intravenous infusion was started as soon as the animals' righting reflexes were lost. Subsequently the animals showed complete anesthesia and muscular relaxation. Following the infusion the animals were kept in warm metabolism cages until recovery was complete. Both animals recovered. The index of recovery was the ability of the animal to stand unsupported. The average oral fatal dose of methanol in dogs is 10 c.c. Thus these animals survived two fatal doses.

One animal received 10 c.c. of methanol dissolved in 1 per cent sodium chloride solution by slow intravenous infusion over a period of forty-three minutes, becoming anesthetic after half of the dose had been injected. This dog recovered in about thirty-six hours. The urine excreted by this animal following injection of the alcohol was of a deep red color, evidently the result of hemolysis.

No evidence of blindness was seen in any of these animals though they were observed for several weeks after recovery.

Ethanol.—Ethanol was administered to two animals orally and to one animal intravenously, as described for methanol. One animal, receiving 15 c.c.

*All doses are expressed in terms of milligram, gram, or cubic centimeter per kilogram of body weight. To avoid repetition the phrase "per kilogram of body weight" will hereafter be omitted.

of ethanol by mouth, recovered, and one, receiving 20 c.c. by mouth, died. A dog receiving 12 c.c. intravenously recovered. The average oral fatal dose of ethanol for dogs is 9.6 c.c.

Chloral Hydrate.—Chloral hydrate was administered in aqueous solution to one animal subcutaneously and to two animals intravenously. The technique employed was the same as that described for methanol. The mean subcutaneous fatal dose of chloral hydrate is 600 mg., whereas 500 mg. are fatal by intravenous injection. Inasmuch as considerably more than this amount was administered, the results may be regarded as significant.

Sodium Bromide.—Sodium bromide in doses of 5.0 Gm. and 7.5 Gm. was administered to each of two dogs in the following manner: The animals were immobilized in plaster of Paris bandages. Under local anesthesia the external jugular vein was exposed and cannulated. An intravenous infusion of 1.5 per cent sodium bromide solution (isotonic) was then given in predetermined dosage. Beyond this point 1 per cent sodium chloride solution was substituted. Although both animals recovered, they showed somnolence for several days and shed quantities of hair; portions of their skin became practically denuded, and deep ulcers developed on various parts of their bodies, particularly over bony prominences. The mean fatal dose of sodium bromide is 3 Gm.

Potassium Arsenite.—Each of five animals was given 2 c.c. of potassium arsenite solution (U.S.P. XI) by mouth. Two of these animals received massive intravenous infusions, the remaining three served as controls. All five animals died. All animals, including those which received massive infusions, lapsed into coma, developed muscular spasms, and died within about the same time. It is concluded, therefore, that massive intravenous infusions had no effect on the course of acute arsenical poisoning. Table I summarizes the results of all experiments previously described.

TABLE I

EFFECT OF MASSIVE INTRAVENOUS INFUSIONS WITH 1 PER CENT SODIUM CHLORIDE SOLUTION ON THE FATE OF DOGS* POISONED WITH FATAL DOSES OF METHANOL, ETHANOL, CHLORAL HYDRATE, SODIUM BROMIDE, AND POTASSIUM ARSENITE

WEIGHT (KG.)	DRUG	ROUTE OF ADMINIS- TRATION	DOSE PER KG. (C.C. OR GM.)	AMOUNT OF FLUID INJECTED		TIME RE- QUIRED FOR INFUSION (HR.)	RESULTS
				TOTAL (C.C.)	% OF BODY WT.		
7.5	Methanol	Oral	20	3,000	40.0	3	Recovered in 72 hours
8.2	Methanol	Oral	20	4,800	53.5	5½	Recovered in 96 hours
16.3	Methanol	Vein	10	5,375	32.8	12	Recovered in 36 hours
15.1	Ethanol	Oral	15	6,600	43.7	4	Recovered in 48 hours
8.8	Ethanol	Oral	20	2,700	30.7	2½	Died in 3 hours
9.25	Ethanol	Vein	12	2,700	29.2	2	Recovered in 24 hours
17.45	Chloral hydrate	Subcutaneous	1	7,500	43.0	2	Recovered in 12 hours
16.6	Chloral hydrate	Vein	0.75	6,025	36.4	4	Died in 5 hours
8.5	Chloral hydrate	Vein	1	4,000	47.0	4	Recovered in 24 hours
5.4	Sodium bromide	Vein	5	2,600	48.1	4	Recovered in 72 hours
11.8	Sodium bromide	Vein	7.5	10,200	92.0	48	Died in 7 hours
8.0	Solution of potassium arsenite	Oral	2	5,100	63.7	5	Died in 12 hours
4.55	Solution of potassium arsenite	Oral	2	2,700	59.4	3	

*These animals excreted in the urine from 20 to 30 per cent of the infused fluid during the course of the infusion, and from 40 to 90 per cent within twenty-four hours.

COMMENT

In our previous communication¹ we discussed the probable mechanism of the antidotal action of massive intravenous infusions in acute barbiturate poisoning. We expressed the opinion that this action is dependent upon the ability of the infused solution to take up a part of the drug, which otherwise would accumulate in toxic concentration in nervous tissues, and to distribute it among the tissues of the body not specifically affected by the drug. When the infused fluids are excreted by the kidneys, the urine contains the neurotoxic poisons in high concentrations, higher than exist in any of the tissues of the body. These considerations apply to neurotoxic poisons because the central nervous system, in contrast to other tissues, does not become hydrated as a result of massive infusions.² The experiments herein described show that the results obtained with barbiturates can be duplicated in the case of three other aliphatic narcotics and sodium bromide, but not in inorganic arsenical poisoning, because arsenic is not primarily a neurotoxic agent and behaves rather as a general protoplasmic poison.

In conclusion, we feel justified in repeating our recommendation for the clinical use of large intravenous infusions in the treatment of alcohol comas, and acute chloral hydrate and bromide poisonings, though not necessarily in the extremely energetic form used in these experiments. Massive infusions should prove less effective or probably not effective in poisoning with general protoplasmic toxins. This treatment is not proposed as a replacement for the rapidly acting analeptics in narcotic poisoning, but rather as an adjuvant. It may be of particular value in cases in which patients do not show a satisfactory response to the analeptic treatment.

REFERENCES

1. Cutting, R. A., and Koppanyi, T.: The Effect of Massive Intravenous Infusions on the Course of Barbiturate Narcosis, *Arch. internat. de pharmacodyn. et de therap.* 60: 395, 1938.
2. Cutting, R. A., Lands, A. M., and Larson, P. S.: Distribution and Excretion of Water and Chlorides After Massive Saline Infusions, *Arch. Surg.* 36: 586, 1938.

CLINICAL CHEMISTRY

PLASMA VOLUME AND PLASMA PROTEIN CONCENTRATION AFTER SEVERE HEMORRHAGE*

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RESEARCHES recently reported^{1, 2} have suggested a possible increase in total circulating plasma protein following shortly after severe hemorrhage. From work previously reported from this laboratory, it is known^{3, 4} that in hydremic plethora extra protein may enter the vascular system from some source in the body, possibly the liver.⁵ Similar changes have been observed with increased plasma volume, following the use of diuretics in the treatment of the edema of circulatory failure and cardiac decompensation.⁶ The present report deals with this problem of protein mobilization in hemorrhage, employing the newer techniques for plasma volume determination.

EXPERIMENTAL

Large dogs were used. Three types of preparation were employed. (A) Dogs without food or water for forty-eight hours prior to hemorrhage. (B) Dogs allowed water but no food twenty-four hours prior to hemorrhage, then given 500 ml. of 0.7 per cent sodium chloride solution by mouth one hour prior to hemorrhage. (C) Dogs allowed water but no food twenty-four hours prior to hemorrhage, with injection into the jugular vein of a volume of 0.9 per cent sodium chloride equivalent to the volume of whole blood withdrawn, immediately after withdrawal.

The hemorrhage from the carotid artery was 25 per cent of the dog's blood volume (as previously determined) within five minutes.

Plasma volumes were determined by the Gibson and Evelyn⁷ techniques; total proteins by the Kjeldahl method; hemoglobins according to Cohen and Smith;⁸ albumins by the Howe,⁹ and later by the Campbell and Hanna¹⁰ methods. Mayo 6 ml. tubes at 3,000 r.p.m. for thirty minutes were used for cell volumes.

RESULTS

A total of 11 dogs was used, 4 in group A, 4 in group B, and 3 in group C. Typical data are presented graphically in Figs. 1 A and B, and Fig. 2, C.

The animals in group A were very dehydrated. This condition is reflected in the posthemorrhage blood picture. There is a slow fall in hemoglobin and per cent of plasma protein. In contrast to these changes, there is a slow rise in plasma volume, and a similar but slightly faster rise in total circulating

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plasma protein (plasma volume/100 \times Gm. protein per 100 ml.). This extra fluid and protein must come from the tissue (or lymph), the protein entering in solution with the water. The changes observed in group A were never so great as for the conditions to be described in B and C, ranging from about 10 to 20 per cent replacement of the total protein removed in hemorrhage.

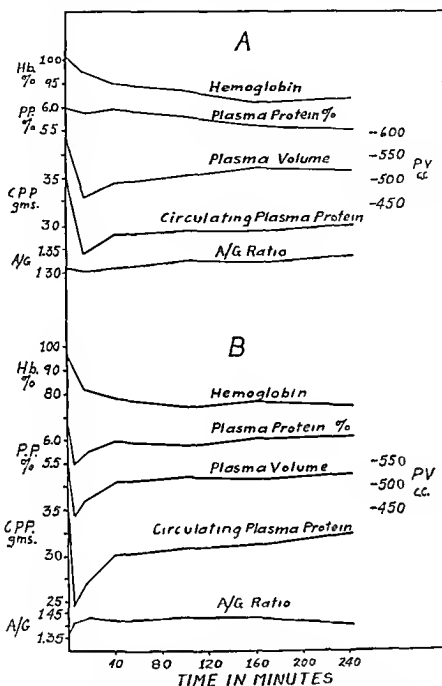


Fig. 1.—A, Hemorrhage after forty-eight hours food and water fast. B, Hemorrhage after twenty-four hours water fast, 500 ml. of 0.7 per cent sodium chloride per os one hour prior to hemorrhage.

In group B the dogs were somewhat dehydrated, but in fair water balance. To this adequate body fluid was added 500 ml. of saline solution, given by mouth, one hour prior to hemorrhage. The one-hour period was selected because of a previous finding³ that injected saline reaches a point of maximum storage in the tissues from sixty to one hundred minutes after intravenous administration. Very little or no diuresis occurred. Thus the body fluids available for posthemorrhage blood volume compensation were at a maximum.

The greater availability of fluid is amply demonstrated in Fig. 1 B. The hemoglobin drops more sharply than in group A. This is also true of plasma protein concentration (per cent). The plasma volume, on the other hand, does not fall off so rapidly, indicating a very rapid inflow of fluid from the inter-

stitial spaces. As in group A, so also here to a more marked degree, this inflowing fluid brings with it considerable amounts of protein, as measured in terms of total circulating plasma protein.

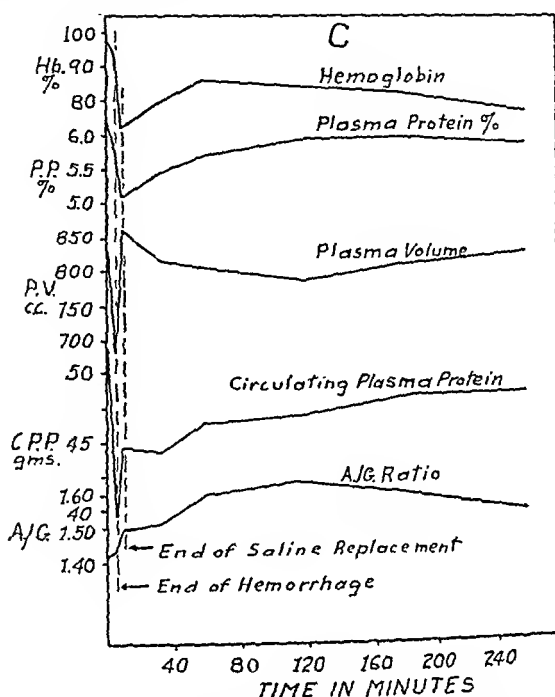


Fig. 2.—C, Hemorrhage after twenty-four hours water fast. A volume of 0.9 per cent sodium chloride equivalent to the volume of blood removed is injected into the jugular vein immediately after hemorrhage.

The animals in group C were in similar fluid balance to those in B, prior to per os saline administration. Immediately after hemorrhage was complete, a volume of 0.9 per cent sodium chloride, equivalent to the volume of blood removed, was injected rapidly into the jugular vein. The initial rapid changes prior to injection of saline were similar to, but somewhat less than, those in group B. After saline injection, however, marked falls in hemoglobin and in per cent plasma protein occurred.

The plasma volume changes were of much interest. From the low value after hemorrhage the volume actually rose above the normal level. However, this condition held only momentarily. As was to be expected from previous data on hemorrhage control,¹¹ the injected saline left the vascular system rapidly and to a considerable extent, resulting in a fall in plasma volume. This condition obtained for upward of an hour, at which time the plasma volume showed a tendency to rise.

During the whole of this period, and more markedly in the first few minutes, extra protein entered the vascular system, as evidenced by the initially rapid, then gradual, rise in total circulating plasma protein.

In all groups, the albumin : globulin ratio rose following hemorrhage.

DISCUSSION

The results here presented must not be confused with those obtained in acute hemorrhage or after plasmapheresis. The degree of hemorrhage used (25 per

cent of the determined blood volume) was not sufficiently severe to upset physiologic equilibrium in the dog. An effort was made to avoid gross evidence of shock. No animals were lost as a result of the procedure. There was, however, some blanching of the buccal membranes, and the heart rate was increased to some extent. Blood pressures were not measured.

Thus the animals were able to affect a rather satisfactory recovery by normal, rapid physiologic response. It is remarkable to note the degree to which this can be accomplished. In one dog, group C, 14.7 Gm. of plasma protein were removed. Within thirty minutes 6.5 Gm. of this had been replaced, and at the end of four hours more than 50 per cent of the plasma protein loss had been taken care of by tissue contribution of storage protein. These values are in line with others obtained in dogs after injections of hypertonic solutions,⁴ where an increase as great as 20 per cent has been observed to occur along with the resultant increase in plasma volume.

The albumin:globulin ratio is of significant interest. This ratio rose in all cases following hemorrhage. The extent of the rise depended upon the degree of total compensation affected in plasma protein, being higher when the increase in total circulating plasma protein was greater. This would seem to indicate, therefore, that the protein entering the vascular system under this condition of stress was composed mainly of albumin. These results are similar to those for hydremic plethora⁴ and those following edema reduction by diuretics.⁶

The exact mechanisms for the changes noted are hard to evaluate. The lymph certainly may serve as one source of this protein and fluid increase. Indeed, evidence has been given for an increased lymphatic flow following similar conditions. The rate of change observed, however, would seem to make it necessary to assume some degree of backflow through the capillaries both of fluid and protein in order to explain the experimental results. Recently, Drinker¹² gave some support to this idea.

The matter of source of protein for the observed increases is not difficult to explain. The liver is known to produce at least part of the protein necessary for plasma protein regeneration. Considering the size of this organ, the slow rate of circulation through it, and the relatively small demand on the whole body made by the replacement of 15 to 20 Gm. of plasma protein, the experimental findings are not untenable. Furthermore, when consideration is given to the total mass of protein in the body, this small amount might easily be accounted for physiologically without being detectable by analytic chemical methods.

It is logical to assume, therefore, on the strength of the data presented, that the body is capable of supplying, rapidly, considerable amounts of protein for the replacement of losses due to hemorrhage, so long as actual gross shock does not intervene. Replacement of fluid depends upon available interstitial supply of water and salts for this purpose.

SUMMARY AND CONCLUSIONS

1. Following subacute hemorrhage in dogs, there is a compensatory movement of fluid from the tissues to the plasma.
2. The degree of compensation depends, in large measure, on the water balance of the body prior to hemorrhage.

3. Proteins enter the vascular system along with the fluid.
4. The protein entering the vascular system is primarily albumin.

REFERENCES

1. Hoitink, A. W. J. H.: Colloid-Osmotic Pressure of Plasma and Composition of Blood After Acute Haemorrhage and After Haemorrhage Followed by Intravenous Injection of 0.9% NaCl Solution, *Arch. néerl. de physiol.* 24: 169, 1939.
- Idem: Colloid-Osmotic Pressure of Plasma and Composition of Blood After Intravenous Injection of Gum-Salt-Solution in Hemorrhage, *Arch. néerl. de physiol.* 24: 194, 1939.
2. Elman, R.: Acute Hypoproteinemia Following Single Severe Hemorrhage in Fasting Dogs, *Am. J. Physiol.* 128: 332, 1940.
3. Calvin, D. Bailey, Smith, A. H., and Mendel, L. B.: A Study of Blood Volume Regulation and Blood Composition in Experimental Hydremia, *Am. J. Physiol.* 105: 135, 1933.
4. Calvin, D. Bailey: Changes in Albumin:Globulin Ratios Following Intravenous Saline Injections, *Am. J. Physiol.* 129: 327, 1940.
5. Madden, S. C., and Whipple, G. H.: Plasma Proteins: Their Source, Production and Utilization, *Physiol. Rev.* 20: 194, 1940.
6. Calvin, D. Bailey, Decherd, G., and Herrmann, G.: Response of Plasma Volume to Diuretics, *Proc. Soc. Exper. Biol. & Med.* 44: 529, 1940.
- Idem: Plasma Protein Shifts During Diuresis, *Proc. Soc. Exper. Biol. & Med.* 44: 578, 1940.
7. Gibson, J. G., Jr., and Evelyn, K. A.: Clinical Studies of Blood Volume; Adaptation of Method to Photoelectric Microcolorimeter, *J. Clin. Investigation* 17: 153, 1938.
8. Cohen, B., and Smith, A. H.: The Colorimetric Determination of Hemoglobin, *J. Biol. Chem.* 39: 489, 1919.
9. Howe, P. E.: The Use of Sodium Sulphate as the Globulin Precipitant in the Determination of Proteins in Blood, *J. Biol. Chem.* 49: 93, 1921.
10. Campbell, W. R., and Hanna, M. I.: The Albumin, Globulins and Fibrinogen of Serum and Plasma, *J. Biol. Chem.* 119: 15, 1937.
11. Erlanger, J.: Blood Volume and Its Regulation, *Physiol. Rev.* 1: 177, 1921.
12. Drinker, C. K.: Quoted from address, New Orleans, March, 1940.

PHENOLPHTHALEIN STUDIES: THE RATIO OF FREE TO TOTAL PHENOLPHTHALEIN IN THE URINE*

ITS INFLUENCE UPON THE "WOLDMAN TEST"

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IN OUR preliminary report¹ which was based on 200 cases (34 normal persons and 166 diseased patients) we found Woldman's test positive in 41 per cent of the normal individuals, in 78.5 per cent of the gastrointestinal cases, and in 82.7 per cent of other types of disease, including jaundice. Our result of positive Woldman tests in 41 per cent of the normal persons is perhaps somewhat higher than the percentage in similar cases obtained by others. On the other hand, however, we also obtained higher positive results in gastrointestinal cases (78.5 per cent) as compared to results by others, e.g., 72 per cent. The high incidence of positive results in our series may be attributed to meticulous interpretation of color changes.

*From the Departments of Pharmacology and Therapeutics and of Medicine, University of Illinois College of Medicine, and the Cook County Hospital, Department of Medicine, Chicago. Assisted by a grant from Phenolphthalein Research, Inc., under the direction of Dr. Bernard Fantus.

Our original observation that the urinary elimination of free phenolphthalein is no test for gastrointestinal ulceration has been confirmed not only by further studies in our laboratories but also by reports from various other clinics. Thus Kremer and others² in a study of 137 cases conclude that the Woldman test may be valuable as an adjunct in diagnosis, but that more must be learned of the physiology involved before it can be considered reliable as a simple test for the determination of gastrointestinal lesions.

Suttenfield,³ from his study of 94 cases, concludes that the proposed phenolphthalein test is of no value in differentiating gastrointestinal disease from other diseases, nor organic from functional intestinal diseases.

In a study of 203 patients Banks⁴ found the test negative in one-fourth of his cases with proved gastrointestinal disease, and positive in one-sixth of the controls. He believes that the test has too wide a range of error to be considered reliable in the diagnosis of gastrointestinal disease.

In his series of 105 individuals Notkin⁵ obtained negative Woldman tests in over 30 per cent of his cases with active gastrointestinal lesions. He also discovered variations from negative to strongly positive in the same patients when the test was repeated at appropriate intervals. He concludes, therefore, that the Woldman phenolphthalein test does not constitute a test for the presence of gastrointestinal lesions.

LeVine,⁶ from a study of 115 cases, concludes that the phenolphthalein test cannot be relied on to prove or disprove the presence of a lesion in the mucous membrane of the gastrointestinal tract.

Slutzky and Wilhelmj,⁷ from experiments on dogs, believe that the variability of their results would seem definitely to exclude the phenolphthalein test as a test for determining the presence of ulceration of the gastrointestinal tract.

Rowland,⁸ however, believes that not enough observations have been done to negate the test completely. In his defense of this test, however, appear certain concepts of phenolphthalein absorption, conjugation, and elimination which are not in accordance with our observations on these facts gained through a study on hundreds of cases during a period of four years. This paper has, therefore, been written with the idea of clarifying some of the more important data about the fate of phenolphthalein in the body: data which may indirectly help in the better understanding of the facts underlying and influencing the Woldman test.

RAPIDITY OF ABSORPTION

Woldman's test is based upon the thesis that, if there is an ulcer in stomach or bowel, ingestion of phenolphthalein in rapidly absorbable form (alcoholic solution) will permit the finding of free phenolphthalein in the urine because the break in the mucosa allows phenolphthalein to enter the circulation more promptly. It is obvious that we cannot compare the results of the quantitative studies done previously by Fantus and Dyniewicz,⁹ and quoted by Rowland, with the results now being discussed. There is a much greater and prompter elimination of phenolphthalein after the alcoholic dose of phenolphthalein than after the same or even larger doses given in capsule form. The time factor in the two studies was also different. In the "Woldman's test" studies the speed of

elimination is measured at two-hour intervals for ten hours, while in the Fantus and Dyniewicz studies interest was centered in the total elimination as shown by collections of twenty-four-hour specimens.

The previous work on phenolphthalein elimination was done after ingestion of doses ranging from 0.10 Gm. to 0.25 Gm. in capsule form. The total elimination was studied for as long as six days. The elimination of from 1.41 to 19.7 per cent was for all doses. That many factors influence the elimination of phenolphthalein in the urine was recognized at that time and set forth in the article, e.g., "the larger the dose the smaller the percentage of the elimination" and "the greater the laxative action the less the elimination." We now are aware of additional causes which influence the amount of phenolphthalein eliminated in the urine, e.g., low elimination in kidney disease and high elimination in "yellow" block (jaundice) and in "white" block (sepsis). There is, of course, a great difference in phenolphthalein elimination when the drug is taken in alcoholic solution or in capsule form. When 100 mg. of phenolphthalein in an alcoholic solution are taken on an empty stomach, we obtain on the average 24 mg. per cent of total phenolphthalein in the stool and 38 and more per cent in the urine. After the ingestion of 300 mg. of phenolphthalein in a capsule, an average of 75.5 per cent of the dose was found in the stool and only 8.9 per cent on the average in the urine. The reason for the apparent discrepancy between our present results and some of the data in our former reports is, therefore, plain.

TABLE I

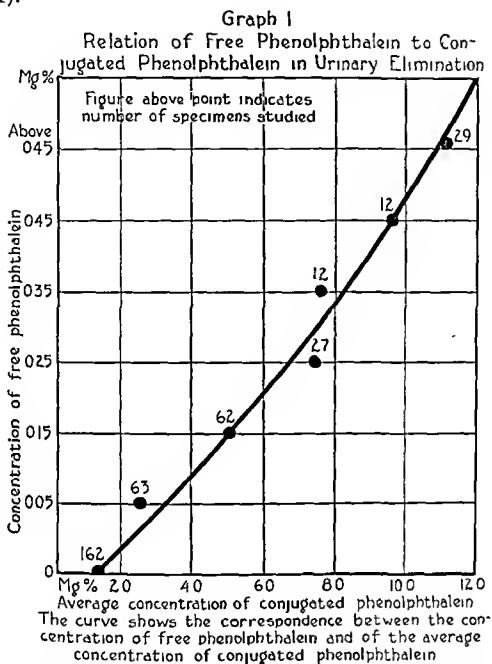
NO. OF SPECIMENS	FREE PHENOLPHTHALEIN PRESENT IN URINE (MG. %)	AVG. CONC. OF CONJUGATED PHENOLPHTHALEIN PRESENT IN URINE (MG. %)
162	0.0	1.31
63	0.05	2.55
62	0.15	5.0
27	0.25	7.4
12	0.35	7.6
12	0.45	9.6
29	More than 0.45	11.0

When one desires to favor absorption of phenolphthalein and does not give it for cathartic action, its administration by the method suggested by Woldman, e.g., in alcoholic solution on an empty stomach, eliminates at least three variable factors: the phenolphthalein is not dependent for solution upon the secretions of the intestines; a more uniform and more rapid absorption occurs, since there is no food interference; and the alcoholic vehicle helps absorption from the stomach. Furthermore, by studying the phenolphthalein elimination at two-hour instead of twenty-four-hour intervals, the amount of phenolphthalein in the urine is much less likely to be influenced by the extent of laxative action, which usually occurs after six hours.

The ten-hour elimination after the alcoholic dose of phenolphthalein frequently exceeds the amount eliminated in six days after the same dose given in capsule form, and consequently the finding of free phenolphthalein is that much more common, as this depends upon two factors: the quantity of total phenolphthalein in the urine, and the bacterial decomposition of the conjugated phenolphthalein.

RELATION OF FREE TO CONJUGATED PHENOLPHTHALEIN

The amount of free phenolphthalein found in the urine is largely influenced by the quantity of conjugated phenolphthalein present (as is shown in Table I and Graph 1).



It must, of course, be understood that every specimen having a concentration, e.g., of 5 mg. per cent of conjugated phenolphthalein does not give a positive test for free phenolphthalein, neither does every specimen having a lower concentration of conjugated phenolphthalein give a negative test for free phenolphthalein. But the general tendency is in this direction (see Graph 1).

Bacteria greatly influence the presence of free phenolphthalein in the urine by causing decomposition of conjugated phenolphthalein to free phenolphthalein on mere standing. Urine containing conjugated phenolphthalein and incubated at 37° C. will show a progressive increase in free phenolphthalein. The same urine kept in the refrigerator or passed through a Seitz filter and then incubated will not show this breaking down of conjugate. Hence prompt testing of the urine is an essential part of the Woldman test.

As previously stated, the data presented mean that "the amount of conjugated phenolphthalein in the urine generally governs the presence or absence

of free phenolphthalein. Hence, any condition which will promote an increased formation or circulation in the blood of conjugated phenolphthalein will lead to the appearance of free phenolphthalein in the urine." It is correctly pointed out, as originally stated by us, that in contrast to the patients only 1 per cent of normal persons show free phenolphthalein when the concentration of conjugated phenolphthalein ranged from 1 to 2 mg. per cent, and that only few normal persons show a concentration of conjugated phenolphthalein of above 5 mg. per cent. It is not the 20 to 40 per cent of positive tests in so-called normal individuals which make the Woldman test unreliable, but the 82 per cent of positive results in diseases other than gastrointestinal. *It is this latter fact of over 80 per cent positive Woldman's tests in nonalimentary tract diseases, which renders this test unreliable for diagnosing an ulcerative lesion in the gastrointestinal tract.* Since ailing individuals are the ones who will be subjected to the test, it is important to note how many patients show free phenolphthalein within the range of 1 to 2 mg. concentration of conjugated phenolphthalein and how many of them have a concentration of conjugated phenolphthalein above 5 mg. per cent. In our study with hospital patients, free phenolphthalein was found in 36 per cent of the specimens when their conjugated phenolphthalein concentration was 1 to 2 mg. per cent, and 46 per cent of the specimens showed a conjugated phenolphthalein concentration above 5 mg. per cent. It is thus clear that the excretion of phenolphthalein in normal persons has actually no bearing in the controversy about the usefulness of the Woldman test in ill patients.

INFLUENCE OF DISEASE

Disease has, however, a most marked influence upon the elimination of free phenolphthalein in the urine. Even certain "mild" affections of the individual, e.g., cold, sore throat, may greatly change phenolphthalein elimination. As previously stated, in kidney disease there may be practically no phenolphthalein in the urine, while in jaundice very little may be found in the stool and as much as 80 per cent in the urine.

The statement "All observers recognized early that cardiovascular renal disease and blood dyscrasias gave a high per cent of positives" can be only partly confirmed by our results. Cardiovascular diseases and blood dyscrasias give a high percentage of positives. Nephritic patients, however, gave in our series the smallest percentage of positives (Table I of preliminary report¹) since they do have a very small elimination of conjugated phenolphthalein. Rowland's interpretation of the positive Woldman's tests in cardiovascular disease, based on the investigations of Portis and Jaffe,¹⁰ who concluded that: "When peptic ulcer is the incidental lesion, it is most often associated with cardiovascular disease," seems logical and should be further investigated. It, however, does not explain the high incidence of positives in the blood dyscrasias and jaundice.

From the findings in our studies we must disagree with the following statements by Rowland: "The conjugation of phenolphthalein with sulfates, glycuronates, or other substances is believed to occur in the liver. This function seems to be suspended in jaundice and free phenolphthalein regularly appears in the urine." It is our experience that in jaundice the amount of conjugated phenolphthalein in the urine is two to four times that of the normal,

a fact which we believe to be the cause of the regular appearance of free phenolphthalein in the urine. Quantitatively the concentration of conjugated phenolphthalein to total phenolphthalein was found to be about 95 per cent in jaundiced individuals. In other words, in jaundice, free phenolphthalein appears regularly in the urine, not because conjugation of phenolphthalein in the liver is suspended (it may even be questioned whether phenolphthalein is conjugated in the liver), but because the urine contains very large amounts of conjugated phenolphthalein.

It is easy to perceive how false positive tests can be obtained in jaundiced cases, when qualitative examinations are performed. This possibility, however, is excluded when quantitative studies are done, as in these the phenolphthalein is extracted by ether and then determined colorimetrically.

Negative Woldman tests may occur when the solution of phenolphthalein is not absorbed from the stomach or intestine, or when absorption is delayed by other than intragastric or intraintestinal factors, since in those instances even a decrease of conjugated phenolphthalein is found in the urine. Work on the factor of absorption is presently going on in our laboratory and promises to give interesting results.

CONCLUSIONS

1. Additional studies on phenolphthalein elimination support the preliminary observation that the higher the concentration of conjugated phenolphthalein in the urine, the more likely the appearance of free phenolphthalein in it.
2. Phenolphthalein elimination after ingestion of 100 mg. of phenolphthalein in alcoholic solution is much greater within ten hours than the elimination after 300 mg. of powdered phenolphthalein in capsule within twenty-four hours.
3. This difference is obviously due to the factor of absorption.
4. Reports in the literature confirm our observation that Woldman's test¹¹ is unreliable as a test for gastrointestinal ulcerations.

We wish to express our thanks to Dr B. Fantus for his valuable suggestions and to Miss E. Redding, R. N., for her technical help.

REFERENCES

1. Steigmann, F., and Dyniewicz, J. M.: Urinary Elimination of Free Phenolphthalein. No Test for Gastro-Intestinal Ulceration, *Am. J. Digest. Dis.* 6: 120, 1939.
2. Kremer, D. N., Shore, P. D., and Wiesel, B. H.: Phenolphthalein as a Test in Gastro-Intestinal Disease, *Am. J. Digest. Dis.* 6: 192, 1939.
3. Suttentfield, F. D.: The Proposed Phenolphthalein Test for Determining the Presence of Gastro-Intestinal Lesions. Results of Ninety-Four Cases, *Am. J. Digest. Dis.* 6: 206, 1939.
4. Banks, B. M., and Barron, L. E.: The Phenolphthalein Test in the Diagnosis of Gastro-Intestinal Disease, *New England J. Med.* 221: 296, 1939.
5. Notkin, L. J., Kirsch, E., and Saul, A.: Note on the Value of Woldman's Phenolphthalein Test for Gastro-Intestinal Lesions, *Am. J. Digest. Dis.* 6: 365, 1939.
6. Levine, Robert, and Kirsner, J. B.: An Evaluation of the Phenolphthalein Test of Woldman, *Am. J. M. Sc.* 198: 389, 1939.
7. Slutsky, B., and Wilhelmj, C. M.: Phenolphthalein as a Test for Gastro-Intestinal Ulceration in the Experimental Animal, *Am. J. Digest. Dis.* 6: 449, 1939.
8. Rowland, V. C.: Editorial, *Am. J. Digest. Dis.* 6: 396, 1939.
9. Fantus, B., and Dyniewicz, J. M.: Phenolphthalein Studies: Elimination of Phenolphthalein, *J. A. M. A.* 110: 796, 1938.
10. Portis, S. A., and Jaffe, R. H.: A Study of Peptic Ulcer Based on Necropsy Records, *J. A. M. A.* 110: 6, 1938.
11. Woldman, Edward E.: A Simple Test for Determining the Presence of Gastro-Intestinal Lesions. A Preliminary Report, *Am. J. Digest. Dis.* 5: 221, 1938.

THE EFFECT OF SULFANILAMIDE ON ACID-BASE BALANCE*

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SINCE the introduction of sulfanilamide in 1937, many important problems concerning its physiologic effects have been adequately studied. However, one significant aspect of the picture remains in a state of confusion; this concerns the effects of the drug on the acid-base balance of the organism. Although it was rather early recognized¹ that administration of the drug produces a condition simulating acidosis—at least in the sense that there is a decrease in the carbon dioxide combining power of the blood plasma—there has not been a complete agreement among different investigators as to the nature of the disturbance in acid-base balance or its cause.

Observations made on patients¹⁻⁵ indicate that administration of sulfanilamide (doses usually 3 to 10 Gm. per day) causes: (1) a decrease in the plasma carbon dioxide combining power, and (2) an increase in urinary carbon dioxide and pH. The same phenomena were observed in dogs by Marshall, Cutting, and Emerson,⁶ the doses being from 0.5 to 2 Gm. per kilogram of body weight per day. Thus it is clear that bicarbonates are being lost from the blood and are being excreted in the urine. The general tendency has been to regard the disturbance in acid-base balance due to sulfanilamide therapy as an acidosis, and to supplement such medication with sodium bicarbonate or lactate. Under such conditions cyanosis is ordinarily absent.

The mechanism which brings about the loss of alkali into the urine has remained obscure, but two explanations have been suggested. Marshall, Cutting, and Emerson propose the following: "It is possible that this (alkaline urine) may be due to lack of reabsorption of bicarbonate and base from the glomerular filtrate, the reabsorption of a large amount of the filtered sulfanilamide interfering with that of the bicarbonate." However, they did not present experimental evidence bearing on this point directly. On the other hand, Hartmann, Perley, and Barnett have, on the basis of clinical observations, taken the view that sulfanilamide exerts its influence primarily on the respiratory mechanism, causing hyperpnea with resultant blowing off of carbon dioxide. They assume this to be followed in sequence by: (1) an increase in blood pH, and (2) a compensatory excretion of alkali by the kidneys. They, therefore, feel that the disturbance in acid-base balance should be properly regarded as a "carbon dioxide deficit type of alkalosis." This statement appears to have created some confusion, since they have also raised the question whether routine alkali administration in conjunction with sulfanilamide is "not only not indicated but definitely undesirable under certain circumstances."

*From the Research Laboratories, Winthrop Chemical Company.
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In view of the questions raised by Hartmann, Perley, and Barnett, it has seemed desirable to present further evidence bearing on the effect of sulfanilamide on acid-base balance, and, if possible, to demonstrate which of the suggested explanations of the disturbance is correct.

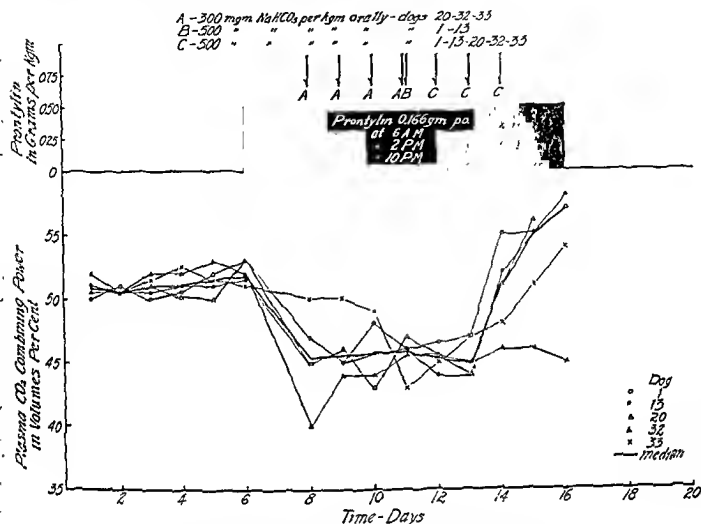


Fig. 1.—Loss of carbon dioxide combining power of plasma caused by sulfanilamide (prontylin) restored by the administration of sodium bicarbonate.

EXPERIMENTAL

I. Demonstration that sulfanilamide acidosis is relieved by alkali.—Five adult dogs were used as subjects. During a preliminary control period of five days the carbon dioxide combining power was determined daily until a normal value was established. Medication consisting of 0.5 Gm. of sulfanilamide per kilogram of body weight was then given in a milk suspension by stomach tube (in three equal doses at eight-hour intervals) daily. The carbon dioxide combining power was determined at the same hour each day until it appeared that no further decrease would occur, then the medication was supplemented with the amounts of sodium bicarbonate indicated in the figure (0.3 to 0.5 Gm. per kilogram per day, in three doses) until the original carbon dioxide combining power was restored. The results of this medication sequence are shown in Fig. 1.

Comment. The median normal carbon dioxide combining power for the group of animals was 51 to 52 volumes per cent. The administration of 0.5 Gm. of sulfanilamide per kilogram per day reduced the carbon dioxide combining power to a median value of 45 volumes per cent, after which no further decrease was noted. The administration of 0.5 Gm. of sodium bicarbonate per kilogram per day rapidly restored this value to 52 volumes per cent or more, but dosages

of alkali ranging (in separate experiments) from 0.1 to 0.3 Gm. per kilogram per day were not sufficient to bring about complete recovery.

II. *Demonstration that sulfanilamide acidosis is prevented by the administration of the proper amount of alkali.* A group of five dogs, including four from the previous experiment, was studied in the same manner as before to determine the normal carbon dioxide combining power. When this value was established, the animals were given 0.5 Gm. of sulfanilamide and 0.1 Gm. of magnesium oxide per kilogram of body weight per day as a single dose. The plasma carbon dioxide combining power was determined daily on a blood sample taken just before medication. This treatment was continued for ten days, and the results are shown in Fig. 2.

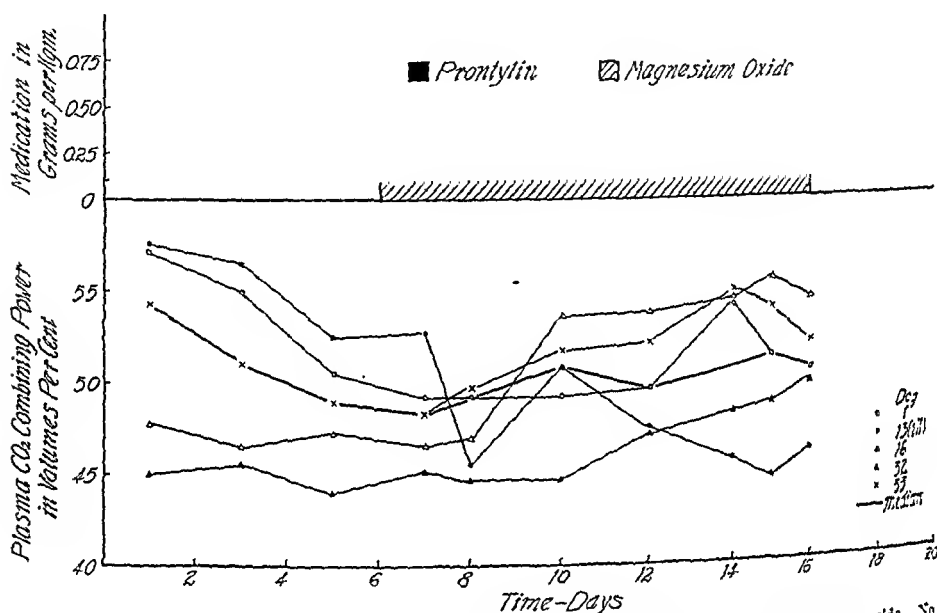


Fig. 2.—Simultaneous administration of sulfanilamide (prontylin) and magnesium oxide. No loss of carbon dioxide combining power results.

Comment. The data represented graphically in Fig. 2 indicate that the normal carbon dioxide combining power for this group of animals is again between 50 and 55 volumes per cent. This value remained practically unchanged during the period of medication, showing that the amounts of alkali and drug given are approximately equivalent physiologically, at least in the dog, and under the conditions of this test.

III. *Demonstration of the order of the changes in the various acid-base factors following sulfanilamide.* A female dog was trained to lie quietly unrestrained on a padded table for long periods of time, was accustomed to retain a urethral catheter and stomach tube, and to permit blood sampling. Respiratory rates and volumes were recorded by means of a pneumograph adjusted to the thorax and connected with a recording tambour, the excursions of which were recorded graphically on a smoked drum. After a satisfactory control period, medication consisting of 0.6 Gm. of sulfanilamide per kilogram of body weight suspended in milk was given by stomach tube (the tube was then removed). Blood samples were taken every thirty minutes and urine samples every fifteen

minutes up to three hours after medication, after which they were taken less frequently. The pH of each sample was immediately taken by means of the glass electrode, the blood determination usually being complete within forty-five seconds after taking the sample, or as soon as a constant reading could be obtained. The urine sample was kept under oil until the blood sample was finished. Respiratory exchange was calculated in arbitrary units; i.e., the rate of

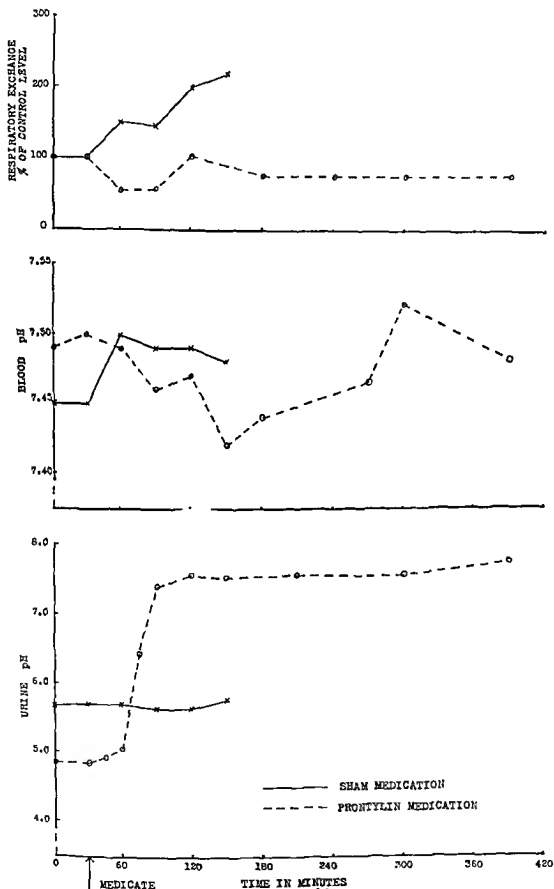


Fig. 3.—Changes in the various acid-base factors following sulfanilamide medication. A, Respiratory exchange is given in terms of per cent of the value recorded during the period preceding medication. B, Figures given for sulfanilamide medication are medians obtained from three separate experiments. C, Figures given for sham medication are averages obtained from two separate experiments.

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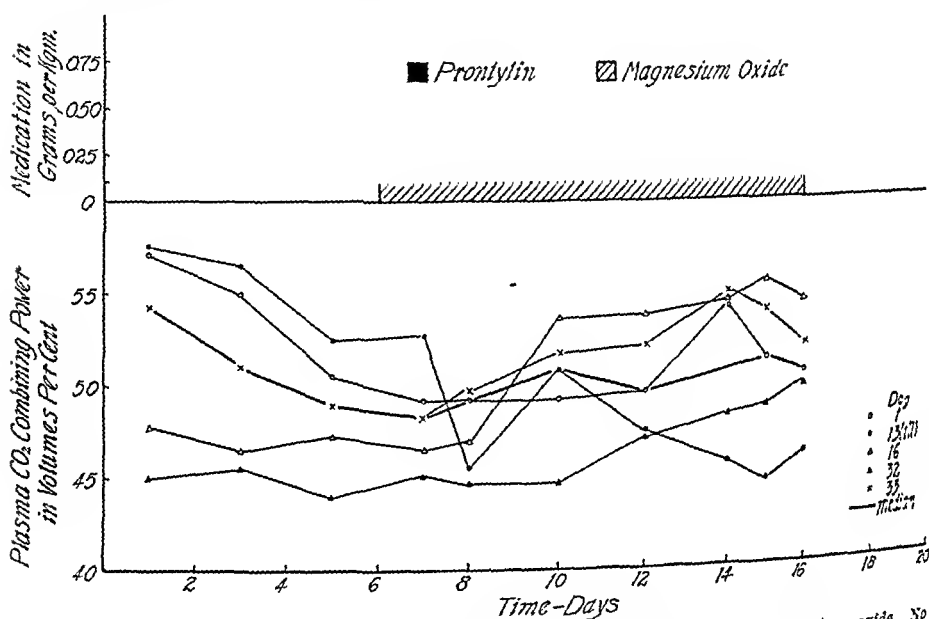


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have been expected if the explanation proposed by Hartmann, Perley, and Barnett were correct. In the third place, we have observed in control experiments hyperpnea and increased blood pH without any change in the pH of the urine. Finally, the blood pH after sulfanilamide tends to *fall* slightly, and to rise later, but only after a respiratory acceleration becomes evident.*

CONCLUSIONS

1. Sulfanilamide causes a disturbance in acid-base balance characterized by loss of alkali from the blood into the urine. This probably has its origin in some type of interference with the reabsorption of bicarbonates from the glomerular filtrate. The phenomenon is, therefore, most accurately designated as a primary alkali deficit due to alkali loss.
2. The alkali deficit which occurs in dogs medicated with sulfanilamide may be compensated by giving alkalis in about the amount of 100 mg. of magnesium oxide or 500 mg. of sodium bicarbonate per 500 mg. of sulfanilamide.

REFERENCES

1. Southworth, H.: Acidosis Associated With the Administration of Para-amino-benzene-sulfonamide (Prontylin), *Proc. Soc. Exper. Biol. & Med.* 36: 58, 1937.
2. Hartmann, A. F., Perley, A. M., and Barnett, H. L.: A Study of Some Physiological Effects of Sulfanilamide, *J. Clin. Investigation* 17: 465, 1938.
3. Strauss, M. B., and Southworth, H.: Urinary Changes Due to Sulfanilamide Administration, *Bull. Johns Hopkins Hosp.* 63: 41, 1938.
4. Brown, A. E., and Bonnick, E. G.: The Use of Sulfanilamide and Prontosil Solution, *Proc. Staff Meet., Mayo Clin.* 12: 377, 1937.
5. Beckmann, W.: The Effect of Sulfanilamide on Electrolyte Metabolism, *J. Clin. Investigation* 17: 537, 1938.
6. Marshall, E. K., Jr., Cutting, W. C., and Emerson, K.: The Toxicity of Sulfanilamide, *J. A. M. A.* 110: 252, 1938.
7. McChesney, E. W., Sprague, K. D., and Barlow, O. W.: The Effects of Sulfanilamide on the Acid-Base Balance of Dogs, *Am. J. Physiol.* 126: 567, 1939.
8. Hartmann, A. F.: Clinical Studies in Acidosis and Alkalosis: Use and Abuse of Alkali in States of Bicarbonate Deficiency Due to Renal Acidosis and Sulfanilamide Alkalosis, *Ann. Int. Med.* 13: 940, 1939.

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*After this paper was submitted for publication it came to our attention that Hartmann has reiterated these same views with new experimental subjects without, however, using any new experimental approach. Our opinion that his views are incorrect remains unchanged. That such is the case is well illustrated by the chart on page 951. Here the urine pH rises immediately after medication, while the serum pH rises slowly (and insignificantly, since within normal limits) over a period of several days. In other words, the "effect" precedes the "cause." The ammonium chloride medication followed by sulfanilamide seems to have little bearing on the question. It is hardly to be expected that during, or immediately after, an overwhelming acidifying dose (enough to neutralize several times all the bicarbonate in the blood plasma) the kidney would begin to excrete bicarbonates. Any bicarbonate escaping reabsorption at this time would naturally be neutralized in the very acid urine.

THE CAPACITY OF THE KIDNEY TO CONCENTRATE URINE IN ACUTE GLOMERULONEPHRITIS*

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IT IS the purpose of this paper to call attention to the infrequency with which attention is paid to the power of the kidney to concentrate the urine in acute glomerulonephritis and to point out what appears to be an error in most of the discussions of this subject. I will review the literature on the subject and report additional cases of acute glomerulonephritis with impairment of ability to concentrate the urine. My previous experience with a number of cases of acute glomerulonephritis has been at variance with the statements in the usual sources concerning the capacity of the kidney to concentrate urine in acute glomerulonephritis. Unless stated to the contrary, acute glomerulonephritis is used here as a designation for the diffuse variety of this disease.

In the important review of the subject of nephritis by Van Slyke and others,¹ I have found no mention of the capacity of the kidney to concentrate when affected by acute glomerulonephritis. Nor do Peters and Van Slyke² discuss this function of the kidney in acute glomerulonephritis. Inasmuch as both papers adequately cover the concentration of the urine by the kidney in chronic glomerulonephritis, one might infer that they do not consider that there is any significant change in this function of the kidney in acute glomerulonephritis.

In this paper the values for the specific gravity of the urine as a criterion of the kidney's concentrating power are based on the work of Addis and Shevky,³ Lashmet and Newburgh,⁴ and Lundsgaard⁵ who used a slightly modified Volhard test. Their results vary slightly, but all are in agreement that with proper technique the kidney should concentrate to a specific gravity of at least 1.025. Addis and Shevky found 95 per cent of normal individuals could concentrate above 1.028, and 100 per cent of normal individuals could concentrate above 1.026.

CASE REPORTS

CASE 1.—W. K., a 17-year-old colored school boy, was admitted to the hospital on September 25, 1938. For many months he had prolonged head colds with severe frontal headaches suggestive of sinusitis. The present illness developed insidiously and began with swelling of the face, malaise, and anorexia one month before admission. One week after onset there was swelling of the abdomen, and finally slight swelling of the feet. He was not aware that he had bloody or smoky urine, or any decrease in urine output. He had increasing edema of the face and swelling of the abdomen and progressive weakness.

Examination showed marked edema of face and trunk, and slight edema of the extremities. The edema was of the hard, brawny type and pitted with difficulty. Subsidizing bilateral otitis media was present; the right ocular fundus showed papilledema, several

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small hemorrhages above the disk, arteries of smaller than normal caliber but no exudate. There was marked pnpilledema on the left. The heart was enlarged to the left, the total transverse diameter being 15 cm., and that of the chest 26 cm. A diastolic murmur was heard along the left border of the sternum and the blood pressure was systolic 158 and diastolic 70. Slight jugular distention, edema of the abdominal wall, slight shifting dullness in the abdomen and slight edema of extremities were present. The spleen descended 2 to 3 cm. below the left costal margin on deep inspiration.

Eight uric examinations were made. All of them showed 2 plus to 4 plus albumin and were acid in reaction. The specific gravity of seven specimens was 1.012, one was 1.015. Only one specimen was positive for blood. Red blood cells were 2,530,000, hemoglobin 34 per cent, white blood cells 13,000, polymorphonuclear cells 88 per cent, lymphocytes 12 per cent. Blood serology was negative.

The patient had fever during his entire stay in the hospital. Both ears drained during most of his hospital course. After one negative culture the blood culture repeatedly showed hemolytic streptococci. The diastolic basal murmur became progressively louder and was associated with a diastolic thrill for the last few days of life. Total blood plasma protein was 7.8 per cent, albumin 3 per cent, and globulins 4.8 per cent. A roentgenogram of the chest showed the total transverse cardiac diameter to be 16 cm., and the chest diameter to be 27.5 cm. On admission the nonprotein nitrogen was 22 mg. per cent, and creatinine 1.7 mg. per cent (frequently found as a normal value in this laboratory). There was a progressive increase in nitrogen retention, which was 85.7 mg. per cent of nonprotein nitrogen, 7.5 mg. per cent of creatinine, and 19 volumes per cent of carbon dioxide combining power the day before death.

The fluid intake was 300 c.c. and 600 c.c. the first and second hospital days, respectively. The output of urine was 300 c.c. and 360 c.c., respectively, the first two days in the hospital. The first twenty-four hours he lost one-half pound, or roughly 250 Gm. During the first week he lost an average of 374 Gm. daily. It is believed that this loss of weight was not all due to fluid loss because of his fever and the extremely small food intake. The specific gravity of the urine was 1.012 on each of the first two days when the output was much diminished. He died on October 18, 1939, twenty-three days after admission.

The clinical diagnoses were acute diffuse glomerulonephritis, acute bacterial endocarditis due to a hemolytic streptococcus on the aortic or pulmonary valves, aortic or pulmonary insufficiency, bilateral otitis media, and hemolytic streptococcal septicemia.

Dr. R. S. Jason, of the Pathology Department, performed a necropsy seven hours after death. The kidneys were enlarged, the combined weights being 705 Gm. The capsules stripped with ease, showing numerous petechial hemorrhages in the cortex. The cut surfaces bulged markedly, the pyramids were dark colored. Microscopic examination of the kidneys showed capsular proliferation, many glomeruli were avascular and showed proliferation of the endothelial cells. Tubular degeneration was marked. An anatomic diagnosis was made of acute thrombo-ulcerative endocarditis of the pulmonary valve; septic emboli to both lungs; diffuse proliferative capsular glomerulonephritis; degeneration of parenchymatous organs and acute splenic tumor; slight dilatation of heart; passive congestion of lungs, liver, spleen, and kidneys; dependent edema; slight osteitis and hydropericardium; and bilateral suppurative otitis media. Cultures from the spleen showed hemolytic *B. coli*; from the lung, *B. coli* and hemolytic streptococci.

Comment.—No concentration tests were done as such. I believe it to be important, however, that the specific gravity of the urine was 1.012 on two days when the output was 300 c.c. and 360 c.c., respectively. That diuresis was likely not a factor in the low specific gravities recorded is further shown by the weight loss of this patient. The first day he lost one-half pound, roughly 250 Gm. During the first week he lost five and one-half pounds, an average of approximately 374 Gm. per day. This was probably not all due to fluid loss because he ate very little, in spite of the fever. On the days when the specific

gravity was 1.012 and the urinary output was very low, the urine contained three-plus albumin. The specific gravity corrected for the albumin would likely have been 1.011 or 1.010.

CASE 2.—H. A., a 51-year-old colored male janitor, was admitted to the hospital on March 29, 1939, and was discharged improved on June 14, 1939. His complaints were shortness of breath, swelling of the feet, legs, and abdomen, and weakness. In February, 1939, he had a disease characterized by fever, generalized aches and pains, and diagnosed as "grippe" by a physician. About February 20 he returned to work but became progressively weaker. Shortness of breath and swelling of his abdomen gradually developed. Swelling of the feet and of the face occurred a few days before admission.

Examination showed him to be pale. He could lie flat without respiratory embarrassment. His heart was of normal size, his mechanism was normal, and his blood pressure was systolic 120 and diastolic 86. Edema of the abdominal wall, especially of the flanks, and two-plus pitting edema of the legs were noted. The fundi oculi were normal.

The urine was cloudy, acid, had a specific gravity of 1.014, and contained three-plus albumin. It was otherwise normal. The blood had red cells 4,570,000, hemoglobin 72 per cent, white cells 13,350, normal differential. Hinton and Eagle precipitation tests were negative.

During the patient's entire stay in the hospital he had no fever and a steady though slow improvement. Weight nineteen days after admission was 180 pounds; two days before discharge, 163 pounds. The weight on admission was probably greater than 183. More than twenty blood pressure recordings were systolic 110 to 120 and diastolic 70 to 80. On March 30, 1939, the venous pressure was 8 cm. of blood. On April 4, 1939, 1,300 c.c. of chyliform fluid was withdrawn from the abdomen, the specific gravity of which was 1.018. On April 12, 1939, the total blood plasma proteins were 4.7 Gm., albumin 2.3, and globulin 2.4. In spite of a four-plus albuminuria the maximal concentration of the urine was 1.015 sixteen hours after abstaining from food and drink. A urea clearance of 9 per cent of maximal clearance and a blood nonprotein nitrogen of 91 mg. per cent were found. On May 6, 1939, the red blood cell count was 2,850,000 and hemoglobin was 40 per cent. On May 12, 1939, the red blood cell count was 3,050,000. On May 18, 1939, urea clearance was 13 per cent of maximal normal; total proteins 4.9, albumin 1.5, globulin 3.4, and the blood nonprotein nitrogen 53.2 mg. per cent. On May 25, 1939, the twenty-four-hour specimen of urine measured 1,100 c.c. and contained 17 Gm. of protein. On June 5, 1939, the nonprotein nitrogen was 31.6 mg. per cent, creatinine 3.0 mg. per cent (normal values in this laboratory often 2). On June 9, 1939, total protein was 6.0 Gm., albumin 3.5, globulin 2.5, urea clearance 15 per cent of the standard clearance.

Clinical diagnoses: Acute glomerulonephritis, ascites, and uremia.

Comment.—Twelve urine specimens contained three-plus to four-plus albumin for the first six weeks in the hospital. Red blood cells were found in the urine only infrequently. Of fifteen urine examinations twelve had a specific gravity of 1.017 or below (1.013 to 1.017); two were 1.018; one was 1.020 one month after admission. In spite of marked edema, ascites, and albuminuria on admission, the specific gravity of the urine was 1.014. On April 20, 1939, in spite of a four-plus albuminuria, the maximal concentration of the urine was 1.018 sixteen to seventeen hours after no food or fluids. It appears from the above data that the impairment of the tubular function was not as extreme as that of the glomerular function. But if the correction is made for the albumin content of the urine, the specific gravity of the urine indicates a greater degree of tubular damage. It is known⁶ that urines which contain a large amount of protein have more than 0.5 per cent. One quantitative estimation of this patient's urine indicated that he had 1.5 per cent. It seems fair.

therefore, to assume that the urines which had three- to four-plus albumin contained approximately 1 per cent albumin. A correction⁷ for the amount of albumin would result in a maximal concentration of 1.015 instead of 1.018.

CASE 3.—O. T., a colored school girl, 15 years of age, was admitted to the hospital on December 6, 1938, with the complaint that four weeks before she was admitted her illness had begun with severe frontal headaches and drowsiness associated with swelling of the face, particularly the eyelids, and feet. The edema subsided during the day and recurred during the night. Seven days before admission visual impairment became so marked that she consulted an ophthalmologist. She gave no history of a previous respiratory or any other type of infection for many months prior to onset of the illness. The urine assumed a darker color during the present illness, but she had not been aware of bloody or smoky urine or any diminished volume. Urinary frequency was four times daily and once at night. She said her face was less swollen than at the beginning of her illness.

Examination showed the face and lids edematous. The blood pressure was systolic 115 and diastolic 80, the heart and lungs were normal, the tonsillar lymph nodes were enlarged, the tonsils were absent, the spleen was not felt, the abdomen was normal. The patient was in a good nutritional state and reflexes were normal. Fundus examination showed marked bilateral papilledema, numerous splinter hemorrhages in both eyes, veins engorged, very slight unevenness of caliber of arteries, a few cotton wool exudates close to the disks, macular areas normal, and two flame-shaped hemorrhages a disk's diameter above right disk.

The urine was acid and cloudy and had a specific gravity of 1.020; it gave a three-plus albumin reaction, and was negative for sugar, acetone, and bile. The sediment showed many long granular and cellular casts, numerous red blood cells, and the benzidine test on the sediment was strongly positive for blood. During the next two months fifteen examinations of the urine were made. A three-plus albuminuria was present on all but two occasions, when it was two plus. Microscopic and/or chemical tests for blood were positive in all but three specimens. Granular casts were found seven times in the fifteen examinations.

The blood on December 8, 1938, showed red blood cells 3.48 million, hemoglobin 54 per cent, white blood cells 4,050, essentially normal differential. On January 3, 1939, red blood cells showed 2.4 million, hemoglobin 28 per cent; the reticulocyte count on January 16 was 4.2 per cent. The red blood cell counts remained from two to three million and the hemoglobin from 28 to 52 per cent. The white blood cell count was low for the first six weeks in the hospital when it went to 11,400. There was a constant leucocytosis from 18,450 on January 20 to 27,800 on January 23 and 22,550 on February 1. On and after February 20 the polymorphonuclear cells were 83 per cent or greater. On December 7, 1938, one day after admission, nonprotein nitrogen was 54.5 mg. per cent, creatinine was 3.0 mg. per cent, carbon dioxide combining power was 53 volumes per cent; on December 12 nonprotein nitrogen was 46 mg. per cent, creatinine was 1.9 mg. per cent; on December 19 nonprotein nitrogen was 28 mg. per cent, and creatinine was 1.4 mg. per cent.

The patient had fever during her entire stay in the hospital; on several occasions her temperature was 104° and 105° F. On December 11, 1938, she was clinically improved, the edema was rapidly disappearing, and she was mentally more alert; the following day her blood pressure was systolic 114 and diastolic 78. Physical examination revealed a productive cough but normal lungs. On December 15, 1938, papilledema decreased, only one flame shaped hemorrhage remained in the right eye; the exudates previously seen had disappeared. Three days later eye grounds were essentially the same. She was given a transfusion of 400 c.c. of citrated blood on January 4, 1939. Two days later the specific gravity of her urine was 1.012; on January 8 the specific gravity of the urine was 1.012.

There were signs of fluid in left chest on January 21, 1939; pericardial friction rub was heard on January 23. The patient was having pain over the precordium, and both the right and left sides of her heart were considered enlarged. Tubular breathing was heard over the left base posteriorly. The question whether this was due to pericardial effusion or pleural or pneumonic disease was raised, but no conclusive decision was reached.

On January 30 two hemorrhages and exudate and papilledema were seen in the left fundus; on January 31 the Rumpel-Leede capillary fragility test was positive. The blood pressure was systolic 140 and diastolic 98. Nonprotein nitrogen was 160 mg. per cent, creatinine was 3.0 mg. per cent, total blood plasma proteins were 6.25, albumin was 2.10, and globulin was 4.15; uremic frost was observed along hair margins. On February 1 total protein was 6.25 Gm. per cent, albumin 2.10 Gm. per cent, globulin 4.15 Gm. per cent, nonprotein nitrogen 160 mg. per cent, creatinine 3.0 mg. per cent; the following day nonprotein nitrogen was 210 mg. per cent and creatinine 3.5 mg. per cent.

Concentration Tests.—(All water and food were withheld fourteen to sixteen hours and the bladder was emptied one to two times before the specimen was obtained for specific gravity estimation.) On December 7, 1938 (the day after admission, four weeks after onset), the specific gravity was 1.019, albumin was three plus; on December 15, specific gravity 1.018, albumin three plus; on December 23, specific gravity 1.017, albumin three plus; January 5, 1939, specific gravity 1.010, albumin two plus; January 8, specific gravity 1.016; January 9, specific gravity 1.012, 90 c.c.; January 10, specific gravity 1.006, 90 c.c.; January 12, specific gravity 1.012, 120 c.c.; January 13, specific gravity 1.012, 150 c.c.; January 14, specific gravity 1.010, 210 c.c.; January 15, specific gravity 1.010, 150 c.c.; January 16, specific gravity 1.007, 180 c.c.

The course grew progressively worse and the patient died February 4, 1939, on the sixty-second hospital day. Permission for necropsy was not obtained.

Clinical diagnoses: Acute glomerulonephritis terminating in uremia, septicemia of undetermined etiology, uremic pericarditis with effusion.

Comment.—This patient probably had impairment of concentrating capacity when she was admitted to the hospital dehydrated, edematous, and with a urine specific gravity of 1.020 and a three-plus albuminuria. There was a gradual decrease in this function until the concentrating capacity was fixed at 1.010 to 1.012 after January 9, 1939. The necessity for making corrections for the albumin content of the urine in specific gravity estimations in concentration tests is again emphasized.

CASE 4.—J. R., a 29-year-old colored clerk, was admitted to the hospital on October 10, 1938, complaining of shortness of breath and a bad cough. The present illness began three weeks before admission with a "head and chest cold" after he had become thoroughly soaked in a rainstorm. He had chilly sensations and fever from the onset of his illness. Several days before admission he developed extreme shortness of breath, edema of lower extremities, headaches, and dizzy spells. Urinary frequency was five to six times in the day and three to four times during the night. Two months before admission he had had gonorrhea which had been treated successfully by a physician.

He exhibited a great amount of unproductive coughing. Examination showed orthopnea. The conjunctivae were markedly blood shot; face and eyelids were "puffy." The fundi oculi showed no pathology. The jugular veins were slightly distended and there were congestive râles in the lung bases. The heart was enlarged to the left anterior axillary line in the fifth interspace. The apical thrust was not forceful. There was a gallop rhythm and pulsus alternans, the heart rate was 140, and the blood pressure was systolic 196 and diastolic 160. There was a small amount of fluid in the abdomen, the liver was tender and extended 4 cm. below the right costal margin. There was a grade two to three edema of both lower extremities up to the knees and slight tenderness in the right costovertebral angle.

The urine on admission showed a specific gravity of 1.026 and contained four-plus albuminuria. It was cloudy and contained numerous red blood cells per low-power field and occasional granular casts. The blood showed red blood cells 4.2 million, hemoglobin 85 per cent, white blood cells 8,300, neutrophils 80 per cent, lymphocytes 19 per cent, and basophiles 1 per cent.

He was given absolute bed rest, digitalis, and sedatives; fluid and salt were restricted. His temperature was within normal limits with the exception of two dif-

ferent days when it reached 100.4° F. rectally. Blood pressure was systolic 196 and diastolic 160 on admission and remained elevated during his stay in the hospital. The weight on admission was 158 pounds; one week later, October 17, 144 pounds; October 24, 139; October 31, 134; November 7, 131; November 14, 129. On October 11 nonprotein nitrogen of the blood was 100 mg. per cent, creatinine 3.0 mg. per cent. On October 15 nonprotein nitrogen was 50 mg. per cent, creatinine 2.3 mg. per cent, carbon dioxide combining power 53 volumes per cent; October 24, nonprotein nitrogen 40 mg. per cent, creatinine 1.3 mg. per cent.

The urine was acid on October 11, had a specific gravity of 1.022, two-plus albumin, six red blood cells per high-power field, and an occasional granular cast. On October 14 the urine was pale yellow, acid, had a specific gravity of 1.009, and three-plus albumin, white blood cells 3 to 4, no red blood cells, one to two granular and hyalin casts; October 15, urine pale, neutral, specific gravity 1.010, two-plus albumin. He developed signs of a pulmonary infarct in the right lower lobe. On October 22 the signs of congestive failure and pulsus alternans had disappeared; urine acid, specific gravity 1.018, two-plus albumin; October 28, uriae acid, specific gravity 1.010, albumin one plus, few red blood cells, and three granular casts per high-power field; October 31, urine yellow, acid, specific gravity 1.017. Hinton and Eagle precipitation tests were negative. He was discharged on November 14, 1938, as improved.

Clinical diagnoses: Acute glomerulonephritis, cardiac dilatation, congestive heart failure, and pulmonary infarct.

Comment.—This man unquestionably had congestive heart failure. The clinical picture otherwise was characteristic of acute glomerulonephritis. I believe that the nonprotein nitrogen value of 100 mg. per cent was too high to be explained on the basis of congestive heart failure, although this probably contributed to the renal failure. The number of red blood cells was also much higher than the few one commonly sees in the urine due to passive congestion of the kidneys. I have concluded that the primary disease was acute glomerulonephritis and that the heart failure was that which is not uncommonly associated with acute glomerulonephritis.

My justification for including this case in this paper is that the urinary specific gravity on admission was 1.026 with a four-plus albuminuria and marked congestive heart failure, both of which factors raise the specific gravity of the urine. It should be noted also that the specific gravity of the urine for the first week after the first specimens gradually reached the level of 1.010. It may be argued that this was in part due to diuresis.

My principal purpose in including this case in this report is to emphasize that there may be nitrogen retention in acute glomerulonephritis with less decrease in the tubular function of concentration than one usually sees in chronic glomerulonephritis.

REVIEW OF LITERATURE

It is stated in commonly consulted sources⁹⁻¹⁹ that the specific gravity of the urine in acute glomerulonephritis is normal or even high. Some of the statements, however, are conflicting. Boyd,^{12, 19} for example, says that acute nephritis is characterized by a urine of high specific gravity and a low urea content. It appears to me that these two statements are incompatible in view of the fact that urea is one of the substances which is normally concentrated to a greater degree than any other substance in the urine. Unless glomerulonephritis is associated with a decreased formation of urea, or there is a unique interference with the capacity of the kidney to concentrate urea alone, neither

of which is true so far as I am aware, it would seem that it is impossible for a high specific gravity of urine to exist with a low urea content. The statement relative to the low urea content of the urine implies either specific impairment of the glomerular filtration of urea, increased tubular absorption of urea, or some impairment of the tubular function of concentration in general. The latter seems a more logical explanation of a low urine content of urea.

The statements in Best and Taylor¹³ also are ambiguous. Referring to the pathology of acute glomerulonephritis they say, "Such a severe reaction must of course, if widespread, cause a high degree of renal insufficiency. The following are among the chief features which may appear: retention in the blood of non-protein nitrogenous products; edema;; and the passage of a small quantity of urine with a high specific gravity." These authors¹⁴ then proceed to define renal insufficiency as a "reduced capacity of the kidney to carry out its functions;" and include a diminished concentrating power as an invariable accompaniment of renal insufficiency.

Bell²⁰ also appears ambiguous or inconsistent in his discussion of the concentrating capacity of the kidney in acute glomerulonephritis. For example, on page 497 he states, "In this publication clinical acute nephritis includes all cases of acute renal disease that exhibit a definite impairment of renal function indicated by retention of nitrogenous products, decreased ability to excrete P.S.P., *inability to form a concentrated urine*, loss of large amounts of protein in the urine, bleeding from the parenchyma of the kidney and severe oliguria and anuria." While on page 523 he says, "The total glomerular filtrate is decreased in amount in severe cases and it is distributed through more tubules than under normal conditions, in which only about one-third of the nephrons are active at any one time. The decrease of glomerular filtrate tends to cause retention of metabolites. The distribution of filtrate through a larger number of tubules where it is exposed to a greater reabsorption tends to produce a concentrated urine."

I offer the following criticism of this concept. In the first place, Bell includes in his definition of acute nephritis an inability to form a concentrated urine. He then proceeds to show why the severe forms of acute glomerulonephritis are able to concentrate their urine. In the second place, this concept appears to be based on two assumptions which may or may not be true. At any rate I know of no proof that they are true. Bell appears to assume that all the glomeruli in acute nephritis are open all the time and never shut down. If this really were the case, it would appear logical to believe that the constant filtration through the glomeruli would not allow the urine to remain in the tubules sufficiently long to concentrate it, inasmuch as it seems likely that the greatest amount of concentration may occur in the tubules when the glomerular capillaries are not open. This concept also fails to take into consideration that all glomeruli and all capillaries of each glomerular tuft are not normally open at any one time. This concept of Bell's further assumes that the tubules associated with these glomeruli are functioning normally, in spite of his statement that in severe cases the glomeruli are practically bloodless from intracapillary and extracapillary exudation and proliferation.

If the latter is so, and it is a common teaching of pathologists, it appears to me that it is not in accord with the facts to assume that the tubules of these nearly bloodless glomeruli can function normally when they too are practically deprived of their blood supply by the glomerular pathology. This statement is based on the work of Hou-Jensen²¹ and Clara²² who have shown that at least 97 per cent of all the arterioles of the medulla (tubules) of the kidney arise from the vasa efferentia.

On the basis of the work of Alving and Van Slyke²³ who showed that concentrating capacity may return many months after return of urea clearance, it seems logical to me to believe that the disturbed tubular function in glomerulonephritis may be due not only to a diminished blood supply from bloodless glomeruli, but also to a specific effect of the toxic agent on the capillaries of the tubule itself.

As a further example of the widespread and firm belief that the kidney in acute nephritis retains its capacity to concentrate the urine well, Albright²⁴ states, in the discussion of a case, that the differential diagnosis rests between acute and chronic nephritis; but that decision against acute nephritis was made because, among other things, the patient failed to concentrate the urine above 1.014 maximum. The case was shown at autopsy to be one of acute glomerulonephritis.

In contradistinction to the above case, which showed a nitrogen retention and a low capacity to concentrate, may be mentioned another case from the same source²⁵ (Case 12, Table I). The onset of illness was three weeks before admission, with marked edema of the extremities, genitals, and face; blood pressure was systolic 150 and diastolic 110; albumin and casts were present in the urine; nonprotein nitrogen was 38 mg. per cent, and dilution and concentration test was 1.002 to 1.032. The tubular function was normal and there was no gross evidence of glomerular failure as observed by the normal blood nitrogen. In other words, it appears that if the capillaries of the glomeruli are sufficiently open to prevent nitrogen retention, the tubules get enough blood to allow them to concentrate the urine normally. And if the glomerular capillaries are sufficiently damaged to cause nitrogen retention, the tubules are sufficiently disturbed functionally to result in impaired concentration.

In spite of Christian and O'Hare's¹⁵ statement regarding the high specific gravity of the urine in acute nephritis, their reported cases would appear to be out of accord with their statement. The interpretation, however, is difficult in view of the fact that we do not know the method by which the urine was collected. For example, Case 1 had fourteen specimens examined over a three-month period. Only three of these are above specific gravity 1.020, and ten specimens range from specific gravity 1.013 to 1.019. One specimen was 519 c.c. for twenty-four hours and had a specific gravity of 1.015. Case 3 had nineteen urine specimens examined over a period of fifty-two days, with a specific gravity ranging from 1.010 to 1.017. Most of these were twenty-four-hour specimens. This patient was followed for six months after this, the specific gravity varying between 1.011 and 1.022. Eighteen months after onset of illness the specific gravity was 1.025. Case 5 had twelve specimens examined, with the specific gravity ranging from

TABLE I

SUMMARY OF TWENTY-TWO CASES OF ACUTE GLOMERULONEPHRITIS FROM THE LITERATURE AND FOUR ADDITIONAL CASES WHICH SHOW IMPAIRED CAPACITY TO CONCENTRATE THE URINE

CASE NO.	AGE SEX	PRECEDING INFECTION	INTERVAL AFTER ONSET OF OBSERVATION	BLOOD PRESSURE	URINE	CONCENTRATION TEST—MAXIMUM	UREA CLEARANCE	BLOOD NITROGEN	EDEMA	OLIGURIA	AUTHOR
1	45 Male	Cellulitis of hand, lymphangitis of arm	At once	185/115	Blood, W.B.C., albumin, casts	1.020 (Addis)	Not done	Blood urea 98 mg. %	Marked	Positive	Addis and Oliver
2	24 Male	Scarlet fever	3 weeks after onset of symptoms	180/105	R.B.C., W.B.C., albumin, casts	1.018 (Addis)	Not done	Blood urea 189 mg. %	Marked	Positive	Addis and Oliver
3	29 Male	Strept. hemolyticus septicemia	3 weeks. Dead 4 weeks after onset	118/50	R.B.C., W.B.C., albumin, casts	1.014 (Addis)	Not done	Blood urea 206 mg. %	Positive		Addis and Oliver
4			6 weeks after onset	170/55 110/65	Blood, albumin	1.001 to 1.010 1.001 to 1.024 (Vollhard)	39 c.c./min., 52% maximal normal	Blood urea 160 mg. %			Rehberg
5	31 Male	Cut finger, axillary abscess	3 weeks 6 weeks	150/100	Gran. and hyalin and cellular casts, albumin, 2 plus	Onset 3/12; 4/2: 1.012 to 1.020; 4/25: 1.017	Not done	Not mentioned	Marked		Longcope
6	26 Male	"Cold"	7 weeks	164/92	4 plus albumin	1.012 to 1.020	Not done	Not mentioned	Positive, feet		Longcope
7	48 Male	Not given	At onset	100/90	Hematuria	1.012 max.; two observations at 3 mo. interval 1.020	12% avg. normal; 80% after 3 mo.	Not given	2 plus		Alyng and Van Slyke

8	43 Male	Not given	1 month	158/90	Hematuria	1.011; still im- paired to 1.012 after 3 mo.	10% avg. nor- mal; normal after 3 mo.	Not given	Absent on admission	Alving and Van Slyke
9	11 Male	Not given	13 months		Hematuria	After 5 yr.; max. 1.018	Normal clear- ance for 5 yr.	Not given		Alving and Van Slyke
10	Male	Boil 3 weeks before admission	Uncertain	140/95	Blood, many R.B.C., W.B.C., albumin, casts	1.006 to 1.014	Not done. No P.S.P. in 1 hr.	N.P.N. 101 mg. %	Positive	Case reports, Mass. General Hospital
11			2 weeks before admission	150/110	R.B.C., W.B.C., albumin, casts	1.002 to 1.032	Not done	N.P.N. 38 mg. %	Marked ex- trimitics, puffiness, over eye- lids and face	Case reports, Mass. General Hospital
12	17 Male	Sore throat	8 days	Not given	Albumin, hematuria, marked	1.013 max.; 1.015 after 3 mo.	Creatinine clearance 14 c.c./min.; clearance had returned to 80 c.c. after 3 mo.	Blood urea 90 mg. %	Not given	Holten
13	19 Female	Erysipelas onset 1/17	3 weeks	Not given	Albumin, hematuria	2/9: 1.017; 2/16: 1.010; normal 6 mo.	Creatinine clearance filtration 9.5 c.c./min.; low 17 mo.	Blood urea 2/9: 140 mg. %; 2/16: 200 mg. %	Not given	Holten
14	5 Male	Scarlet fever	Immediately after onset			1.010 on admis- sion; normal after 41 days	Slightly impaired	Blood urea 90 mg. %		Holten

TABLE I—Cont'd

CASE NO.	AGE SEX	PRECEDING INFECTION	INTERVAL AFTER ONSET OF OBSERVATION	BLOOD PRESSURE	URINE	CONCENTRATION TEST—MAXIMUM	UREA CLEARANCE	BLOOD NITROGEN	EDEMA	OLIGURIA	AUTHOR
15	27 Female	Sore throat	Immediately after onset			1.012 maximal normal 3 mo.	Creatinine 65 c.c./min. Normal, this person 115 c.c.; normal 4 mo.	Blood urea 50 mg. %			Holten
16	18	Sore throat	10 days	Normal throughout		1.010 to 1.016 13 days after onset; 1.009 to 1.017 20 days after; 1.000 to 1.023 40 days after onset	Not done	N.P.N. 52 mg. % 13 days after onset; 46 mg. % 20 days after onset	None		Donath
17		Sore throat	14 days	Normal	Blood, albumin, casts	1/14: 1.014 to 1.018; 1/21: 1.012 to 1.020; 1/26: 1.010 to 1.025; 2/1: 1.003 to 1.024	Not done	1/14: N.P.N. 62 mg. %; 1/21: N.P.N. 38 mg. %			Donath
18	26	Sore throat 7/11/29	8 days	Normal	R.B.C., pus cells, albumin, casts	7/19: 1.010 to 1.016; 8/6: 1.012 to 1.023		N.P.N. 36 mg. %	None		Donath
19	Adult			160	Blood, albumin 4 %	1.015 maximum					Vollhard and Fahr
20	Adult			144	Blood, albumin 0.25 %	1.018					Vollhard and Fahr

21	Adult					1.013 maximum	N.P.N. 80 mg. %		Yes 350 c.c./24 hr.	Vollhard
22	27 Male		Normal	Marked hematuria		1.010 fixed			Yes 200 c.c./14 hr.	Vollhard
W.K.	17 Negro Male	Frequent sore throats, sinusitis, otitis media	1 month	Hematuria, albu- minuria		1.010 to 1.012	Not done	N.P.N. 23 mg. % on admission	Yes 300 c.c.; 360 c.c. 2 days	Gregory
H.A.	51 Negro Male	"Grippe"	2 to 4 weeks	Slight hematuria, albumin, casts		1.018; 4 plus albuminuria	9% of maximal normal	N.P.N. 91 mg. %	At onset	Gregory
O.T.	15 Negro Female	None known	4 weeks	Marked hematuria, albumin, casts		Decreased from 1.017 on Dec. 23 to 1.010 and 1.012 on Jan. 9	Not done	N.P.N. 54 mg. % on admis- sion; 12/19: 28 mg. %; 2/2: 210 mg. %	Not noted	Gregory
J.R.	29 Negro Male	"Head and chest cold"	Less than 3 weeks	Hematuria, albumin, casts		1.004 to 1.015, 3/27/39	33% maximal normal on May 8	N.P.N. 10/10: 100 mg. % on ad- mission; 10/15: N.P.N. 50 mg. %; 10/24: N.P.N. 40 mg. %	Present on ad- mission	Gregory

1.008 to 1.014. Eleven months after onset of illness, the specific gravity was 1.019; forty-two months after, it was 1.028. The last two specimens were single-voided specimens. In the absence of specific knowledge of the method of collecting the specimens, one cannot state too definitely that the figures are evidence of a diminished concentration capacity of the kidneys. It may be fairly assumed that there was some uniformity of collection of specimens, and the trend upward in specific gravity, with the passage of time, may possibly be interpreted as evidence of previous impairment of concentration.

Difficulty also arises in connection with Christian and O'Hare's cases in the matter of deciding to what extent diuresis may have been a factor in the low specific gravities reported. The fact, however, that Case 1 had urinary specific gravities of 1.012, 1.014, and 1.015, while at the lowest recorded weight of 58.2 kg., would indicate that diuresis was probably not the cause of the low specific gravity. In Case 3, the specific gravities of 1.010 and 1.014 were also on days when the lowest weight of 51.2 kg. was recorded. This fact appears to render diuresis an unlikely explanation for the low specific gravity. Diuresis was probably not the explanation of the low concentrations of the urine in Case 5, as evidenced by specific gravity of 1.008 and 1.011, with the highest weight of 62.8 kg. on admission, which fell in eighteen days to 52.8 kg., the lowest recorded; the urine, however, continued to show specific gravities of 1.009, 1.010, and 1.011 at the lowest weight recorded.

The report of Murphy, Grill, and Moxon²⁶ is also difficult to interpret accurately regarding the concentrating capacity of the kidney in acute glomerulonephritis. It is of suggestive importance, however, that in twenty patients who became chronic cases the second specific gravity recorded in each case (one to four months after the first) was lower than that on the first study. On the other hand, the specific gravity in ten patients who recovered, the second observation being one to four months after the first, the specific gravity at the second examination, with one exception, was higher than that of the first examination. It seems fair to assume some uniformity in the manner in which the first and second urines were collected in each instance.

I have been able to find but few instances in which the concentrating capacity of the kidney in acute glomerulonephritis has been studied by any of the concentration tests. Most of the reports on acute glomerulonephritis, therefore, are of little value to the purposes of this article. Yet in one year's time I have observed four cases of acute glomerulonephritis with impairment of concentration capacity on a relatively small medical service.

While the general sources cited indicate that the capacity of the kidney to concentrate is unimpaired in acute nephritis, most of the special works on the kidney do not agree with this viewpoint. I have found twenty-one cases of acute nephritis with such impairment found by ten different observers in the relatively recent literature. These cases are summarized in Table I, together with the four additional cases described here.

Addis and Oliver²⁷ reported three cases of acute glomerulonephritis with unquestioned impairment of concentration (Cases 1, 2, 3, Table I). Rehberg²⁸ studied six cases of acute glomerulonephritis from which he concluded that the tubular function may also be affected in this stage of the disease. He gives data

from only one case, however (Case 4, Table I). Longcope²⁹ studied nine cases of acute glomerulonephritis, but only two of these had studies directed at the power of the kidney to concentrate the urine; both of these showed impaired concentration (Cases 5 and 6). The Addis technique was observed in one of these and the volume of the twelve-hour night urine was found normal. The low specific gravity, therefore, was not due to diuresis.

Volhard and Fahr³⁰ state that the concentrating capacity is impaired in acute glomerulonephritis as follows: "The lower the specific gravity of the urine, with small amounts of urine, the greater the kidney damage"; and "In general, in the more severe forms of acute glomerulonephritis the rule is the concentrating capacity of the kidney suffers; and indeed the more so, the more intensively and extensively the glomeruli are diseased." They give very brief summaries of two cases which demonstrated this feature (Cases 19 and 20).

Although Volhard states in another place³¹ that the concentrating capacity of the kidney was only slightly or not at all disturbed in acute glomerulonephritis, the two cases which he cites had markedly impaired capacity to concentrate the urine (Cases 21 and 22).

Donath³² has reported three patients with acute glomerulonephritis who showed impaired concentrating capacity of the kidney (Cases 16, 17, 18). While in two patients there was very slight nitrogen retention and in one there was none, all three had impaired concentration at times when the blood nitrogen was normal. There was also impairment of the capacity to dilute the urine. Both types of impairment persisted after red blood cells, casts, and albumin disappeared.

Holten³³ studied 25 patients with acute glomerulonephritis with the Volhard-Strauss concentration technique and the creatinine clearance test of Holten and Rehberg.³⁴ In this group there were four instances (Cases 12 to 15) of either moderate or severe impairment of concentration, and two other instances of slight impairment. In all instances in which elevation of blood nonprotein nitrogen occurred, there was diminished capacity to form a concentrated urine. In one patient the filtration rate returned to normal before the concentration test. This was also shown by one of the patients of Alving and Van Slyke. The possible significance of this will be discussed in connection with the work of Alving and Van Slyke.

Holten³³ is well aware, of course, that the concentrating capacity of the kidney may be retained in acute glomerulonephritis, but states further that he has never seen it retained in patients with serious impairment of the filtration rate (below 10 c.c. per minute). He cites Volhard's explanation for the maintenance of the concentrating capacity of the kidney in acute nephritis, namely, that the tubules are able to excrete solids to a normal extent and the glomeruli are unable to excrete water.

Alving and Van Slyke²³ have shown results which are interesting and pertinent to the present paper in their comparative values of the urea clearance and concentration tests in Bright's disease. In three transitory cases of glomerulonephritis (hematuria, proteinuria, and initial edema left no doubt that each patient had an acute nephritis) both urea clearance tests and concentration tests

were normal throughout. They also had three cases (Cases 7, 8, 9) of acute glomerulonephritis which had impairment of both the urea clearance tests and concentration tests. Their fourth case had an original urea clearance of 12 per cent of the average normal and a maximal concentrating capacity of 1.012. Three months later the clearance test had risen to 80 per cent of average normal, but the concentration capacity by both the Addis-Shevky and Lashmet-Newburgh tests had risen only to from 1.019 to 1.020. Later this also returned to normal. Their fifth case also showed a marked lag in the return to normal of concentration capacity after the clearance had returned to normal. Three months after onset the urea clearance was normal, but the Addis test showed a maximal concentration of 1.012 which later returned to normal. Their seventh case did not have a return to the normal concentration capacity until five years after the urea clearance was normal.

Lyttle³⁵ calls attention to impairment of concentration in severe cases of acute nephritis, but does not give any cases to demonstrate it.

Goldring, Clarke, and Smith³⁶ showed that at low plasma levels of phenol red, about 94 per cent of the dye clearance is accomplished by tubular activity in the normal human kidney. On the basis of this work Goldring and Smith³⁷ have studied the glomerular and tubular functions of the kidney with inulin and phenol red clearance ratios in patients with various stages of glomerulonephritis. In general, they have found in the earlier stages of the disease that the phenol red-inulin clearance ratios tend to maintain the normal value, which is above 3.0. They have interpreted this as suggesting that injury of glomeruli and tubules progress in a parallel manner. In the more advanced cases, however, this ratio fell as low as 0.6, indicating almost complete loss of the capacity of the tubules to excrete the dye. In a few patients observed early in the acute stage of the disease, the phenol red-inulin ratio was 210 per cent of the average normal figure. In interpreting this they have suggested the possibility of dilatation of the efferent glomerular arteriole which would give a better blood supply to the tubules, and at the same time lower the effective filtration pressure in the glomeruli. They have also suggested that permeability of glomerular capillaries may be reduced without obstructing the blood flow.

COMMENTS

It may be readily understood from the anatomic relationships of the blood supply to the tubules, which is derived from the efferent glomerular arterioles, that the tubular function of concentration may suffer when the glomerular tufts are so damaged as to reduce the urea clearance to low levels. It is difficult to understand, however, why the tubular function may be impaired long after glomerular function has returned to normal. As a tentative explanation, I offer that the tubular capillaries in some instances are damaged to a great degree at the same time the glomerular tufts are damaged, the latter recovering more quickly.

It is certain that tubular function is impaired in acute glomerulonephritis. This is shown by the impaired capacity to concentrate urine, which is illustrated by the cases which have been summarized in Table I. It is further supported by the results of Goldring and Smith³⁷ which indicate that glomerular and tubular damage proceed in a parallel fashion.

On the basis of the reports by Holten³¹ and Alving and Van Slyke²³ of cases of acute glomerulonephritis which showed a return of urea or creatinine clearances to normal levels long before the return of the concentrating capacity, I have made the suggestion that tubular function may be impaired in acute glomerulonephritis not only because the tubular blood supply is disturbed in the diseased glomeruli, but also because the tubular capillaries themselves may possibly be the seat of specific pathology, or that specific damage to the tubular cells may account for it. The specific tubular capillary or cellular damage theory gains some support in the results of Goldring and Smith which showed in advanced cases of nephritis the reduction of the phenol red-inulin clearance ratios to such low levels as 0.6.

It may be seen from Table I that when there is sufficient glomerular damage in acute nephritis to cause nitrogen retention, there is sufficient tubular damage to interfere with the concentrating function of the kidney. I have found no exception in the literature to this. It has been emphasized that it is necessary to correct specific gravity values when the urine contains large amounts of protein.

It is my belief that impairment of concentrating capacity of the kidney will be found in most of the severe cases of acute glomerulonephritis if properly evaluated.

SUMMARY

The literature has been reviewed and twenty-two cases of acute glomerulonephritis with impaired capacity to concentrate the urine have been found and summarized in tabular form, together with four additional cases of acute glomerulonephritis which showed impairment of this function. Certain theoretical considerations have been discussed.

CONCLUSIONS

It is established that the capacity of the kidney to concentrate urine in acute glomerulonephritis may be impaired.

Most, if not all, cases with sufficient glomerular damage to cause blood nitrogen retention will show impaired ability to form a concentrated urine.

Nitrogen retention may occur in acute glomerulonephritis with less impairment of concentration than is usually observed with nitrogen retention in chronic glomerulonephritis.

The necessity for making corrections in the specific gravities of urines which contain large amounts of protein is emphasized.

The cause of impaired tubular function in acute glomerulonephritis is due, at least in part, to the interference with tubular blood supply produced by the proliferative and exudative processes in the glomeruli.

There is evidence that there may be a specific localized effect of the toxic agent of acute glomerulonephritis on the capillaries or cells of the tubules.

REFERENCES

1. Van Slyke, D. D., Stillman, Edgar, Moller, Eggert, Ehrlich, W., McIntosh, J. F., Leiter, L., Mackay, E. M., Hannon, R. R., Moore, N. S., and Johnston, Christopher: Observations on the Courses of Different Types of Bright's Disease, and on the Resultant Changes in Renal Anatomy, *Medicine* 9: 257, 1930.
2. Peters, J. P., and Van Slyke, D. D.: *Quantitative Clinical Chemistry, Interpretations*, Baltimore, 1932, Williams & Wilkins Co., Vol. 1, p. 354.

3. Addis, T., and Shevky, M. C.: A Test of the Capacity of the Kidney to Produce a Urine of High Specific Gravity, *Arch. Int. Med.* 30: 559, 1922.
4. Lashmet, F., and Newburgh, L. H.: The Specific Gravity of the Urine as a Test of Kidney Function, *J. A. M. A.* 94: 1883, 1930.
5. Lundsgaard, C.: Modification of the Concentration Dilution Test, 1920 (Unpublished). Quoted by Alving, Alf S., and Van Slyke, D. D.: The Significance of Concentration and Dilution Tests in Bright's Disease, *J. Clin. Investigation* 13: 969, 1934.
6. Mattice, Marjorie R.: Chemical Procedures for Clinical Laboratories, Philadelphia, 1936, Lea & Febiger, p. 220.
7. Kolmer, John A., and Boerner, Fred: Approved Laboratory Technique, ed. 2, New York, 1938, D. Appleton-Century Co., p. 132.
8. Lashmet, F. H., and Newburgh, L. H.: An Improved Concentration Test of Renal Function, *J. A. M. A.* 99: 1396, 1932.
9. Emerson, Charles Phillips: Textbook of Medicine, Philadelphia, 1936, J. B. Lippincott Co., p. 976.
10. Cecil, Russel L.: A Textbook of Medicine, Philadelphia, 1938, W. B. Saunders Co., p. 946.
11. Meakins, Jonathan Campbell: The Practice of Medicine, St. Louis, 1938, The C. V. Mosby Co., p. 1162.
12. Boyd, William: A Textbook of Pathology, Philadelphia, 1938, Lea & Febiger, p. 642.
13. Best, Charles H., and Taylor, Norman B.: The Physiological Basis of Medical Practice, Baltimore, 1937, William Wood & Company, p. 620.
14. *Ibid.*, p. 634.
15. Christian, H. A., and O'Hare, J. P.: Oxford Medicine, New York, Oxford University Press, Vol. III, Part II, p. 668.
16. Todd, J. C., and Sanford, A. H.: Clinical Diagnosis by Laboratory Methods, Philadelphia, 1936, W. B. Saunders Co., p. 198.
17. Musser, J. H.: Internal Medicine, ed. 3, Philadelphia, 1938, Lea & Febiger, p. 552.
18. Osler, Sir William: The Principles and Practice of Medicine, New York, ed. 13, revised by H. A. Christian, D. Appleton-Century Co., p. 865.
19. Boyd, William: The Pathology of Internal Diseases, ed. 2, Philadelphia, 1935, Lea & Febiger, p. 433.
20. Bell, E. T.: The Pathology and Pathogenesis of Clinical Acute Nephritis, *Am. J. Path.* 13: 497, 1937.
21. Hou-Jensen, H. M.: Die Verästelung der Arteria renalis in der Niere des Menschen, *Ztschr. f. d. ges. Anat. (Abt. 1)* 91: 1, 1929.
22. Clara, M.: Anatomie und Biologie des Blutkreislaufes in der Niere, *Arch. f. Kreislauforsch.* 3: 42, 1938.
23. Alving, Alf S., and Van Slyke, D. D.: The Significance of Concentration and Dilution Tests in Bright's Disease, *J. Clin. Investigation* 13: 969, 1934.
24. Albright, Fuller: In Discussion of Case Records of the Massachusetts General Hospital, edited by T. B. Mallory, *New England J. Med.* 217: 274, 1937.
25. *Ibid.* 213: 1030, 1935.
26. Murphy, F. D., Grill, John, and Moxon, G. F.: Acute Diffuse Glomerular Nephritis, *Arch. Int. Med.* 54: 483, 1934.
27. Addis, Thomas, and Oliver, Jean: The Renal Lesion in Bright's Disease, New York, 1931, Paul B. Hoeber, pp. 51, 77, 81, 229.
28. Rehberg, P. B.: The Kidney in Health and Disease, by Berglund, Hilding, Meder, Grace, Huber, G. C., Longcope, W. T., and Richards, A. N., Philadelphia, 1935, Lea & Febiger, p. 86.
29. Longcope, W. T.: *Ibid.*, p. 362.
30. Volhard, F., and Fahr, T.: Die brightsche Nierenkrankheit, Berlin, 1914, Julius Springer, pp. 119, 125.
31. Volhard, F.: Handbuch der inneren Medizin. Nieren und ableitende Harnwege, edited by von Bergmann, G., von Staehelin, R., and others, Band VI, Teil 2, Berlin, 1931, Julius Springer, p. 1510.
32. Donath, Julius: Ueber postinfektiöse Schädigungen der Nierenfunktion, *Wien. klin. Wchnschr.* 43: 591, 1930.
33. Holten, Cai: Observations Regarding Kidney Function Tests in Acute Nephritis, *Am. J. M. Sc.* 185: 789, 1933.
34. Holten, C., and Rehberg, P. B.: Studies on Pathological Function of Kidneys in Renal Disease, Especially Bright's Disease, *Acta med. Scandinav.* 74: 479, 1931.
35. Lyttle, John D.: The Treatment of Acute Glomerulonephritis in Children, *Bull. New York Acad. Med.* 14: 212, 1938.
36. Goldring, W., Clarke, R. W., and Smith, H. W.: The Phenol Red Clearance in Normal Man, *J. Clin. Investigation* 15: 221, 1936.
37. Goldring, W., and Smith, H. W.: Differentiation of Glomerular and Tubular Function in Glomerulonephritis, *Proc. Soc. Exper. Biol. & Med.* 37: 180, 1937.

BLOOD CHEMISTRY DETERMINATIONS IN PERNICIOUS ANEMIA*

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THE object of this paper is to report the blood chemistry determinations on 15 patients with pernicious anemia in a state of relapse. These were carried out with the hope that some alteration in the normal constituents of the body metabolism might be revealed which would aid in elucidating the obscure nature of this disease.

Minot and Murphy discovered that patients with pernicious anemia improved when given liver. Castle and his associates showed that patients with untreated pernicious anemia lacked in their gastric secretions an essential thermolabile substance termed the intrinsic factor. Sturgis and Isaacs later demonstrated the effectiveness of pig stomach in the treatment of pernicious anemia, and the similarity of this active principle to that found in normal human gastric juice. Both of these factors differ from the substance found in the liver, which is able to stimulate the bone marrow. It is assumed that the intrinsic factor found in the gastric juice combines with an extrinsic factor present in beef to form the active principle which is stored in the liver and is able to stimulate the bone marrow to normal activity. Dakin and West have demonstrated a substance in liver which is probably a dipeptide of β -hydroxyglutamic acid and L- γ -hydroxyproline which they believe to be the active principle in the liver. It was for some time believed that the extrinsic factor was the vitamin B₂ or G, which has been shown to be riboflavin. Mixtures of riboflavin and normal gastric juice, when administered to patients with untreated pernicious anemia, failed to elicit a reticulocyte response, and it is doubtful whether riboflavin is the extrinsic factor.

Even though we have a treatment for pernicious anemia, and have made progress in locating the site and the nature of the active principle, there is still much to be learned about the nature of the disease, and the active principle involved in its production. It is with this hope that we present the following data. All the determinations have been done in the laboratories of the Presbyterian Hospital of Chicago, and references to the methods used will be found in the bibliography.

Table 1 contains all the results on lipid metabolism we were able to obtain. We are cognizant of the fact that Peters and Van Slyke, in the chapter on lipoids, express the opinion that increased lipoids in the various types of anemia is merely an expression of a general reaction to loss of blood and hemoglobin from the circulating fluid. This reaction is said to consist of an increase

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of fat and fatty acids in the blood, and a simultaneous deficiency of the phospholipids and cholesterol. A summary of the results reveals the following:

1. Thirteen total lipid determinations done on 9 patients showed values twice that of normal or higher.

2. Sixteen total cholesterol determinations done on 12 patients demonstrated normal values in 6, moderately decreased in 9, and markedly decreased in 9. The distribution between the free and esterified form was essentially normal.

3. Lipoid phosphorus and lecithins were markedly diminished in 16 determinations done on 12 patients.

4. Of 11 fatty acid determinations done on 8 patients, 7 were much below minimal values, and 4 were below normal values.

TABLE I

CASE NO.	HB.	R.B.C.	W.B.C.	TOTAL LIPIDS (GM. %)	TOTAL CHOLESTEROL	FREE CHOLESTEROL	CHOLESTEROL ESTERS	LIPID PHOSPHORUS	LECITHINS	FATTY ACIDS	MATERIAL USED
						MG. PER 100 C.C.					
1	35	1.540	5.6	1.2	158.7	54.3	104.4	5.2	130		Plasma
2	40	1.170	3.7	1.29	125.7	71.4	54.3	3.3	82.5		Plasma
3	40	1.480	4.8		116	50	66	5.4	129		Plasma
4	31	1.117	3.6	2.8	119	61	58	6.3	156		Plasma
5	58	2.350	3.6		261	92	169	12.1	303	419	Plasma
5	58	2.350	3.6		253	110	143	11.6	289		Serum
6	55	2.080	5.8	1.89	222	128	94	9.2	229	338	W.B.
6	55	2.080	5.8	1.43	234	118	116	7.4	185	337	Serum
6	55	2.080	5.8	1.43	205	104	101	7.3	184	336	Plasma
7	55	1.620	5.4	1.50	178	84	94	8.4	211	133	Serum
7	55	1.620	5.4	1.50	222	127	95	7.9	179	142	Plasma
8	56	2.320	5.6	1.69	157	67	90	8.0	200	256	Plasma
9	68	2.800	8.7	1.53	174	65	109	12.7	318	285	Plasma
10	28	1.280	3.2	1.73	161	73	88	8.2	205	244	Plasma
11	36	1.280	4.3	1.53	180	81	99	14.9	372	163	Plasma
12	40	1.800	3.8	1.29	93	43	50	6.3	157	211	Plasma

TABLE II

CASE NO.	HB.	R.B.C.	W.B.C.	AMINO ACIDS	Ca	P	N.P.N.	UREA N	URIC ACID	CREATININE	SUGAR	CO.	NaCl
1	35	1,54	5.6	11.7									
2	40	1,17	3.7	10.1									
3	40	1,48	4.8	5.9									
4	31	1,12	3.6	5.7									
6	55	2,08	5.8	4.8	10	4	27.8	10	3	1.2	93	69.9	495
8	56	2,32	5.6		10	3.9	25	11	2.7	1.1	113		550
9	68	2,80	8.7		8.7	3.1	29				55		530
10	28	1,28	3.2		8.4	3.5	44.4	23.5	3.6				
13	30	0.98	4.8	21.5									

Table II shows the results of a smaller series of blood chemistry determinations done on 9 patients.

1. Amino acid determinations done on 6 patients showed normal values in 3, slightly elevated values in 2, and a value of 21 mg., which is nearly three times normal, in one.

2. Calcium and phosphorus determinations on 4 patients were normal as were two creatinine and three uric acid determinations.

3. Four nonprotein nitrogen determinations were done, 3 of which were normal and one slightly elevated. Two urea nitrogen determinations were normal, and the third, the same patient who showed the increase in the nonprotein nitrogen, showed also an increase in the urea nitrogen.

4. Two glucose determinations, as well as two carbon dioxide, and three sodium chloride determinations were normal.

TABLE III

CASE NO.	HB.	R.B.C.	W.B.C.	ROSE BENGAL* (%)		INORGANIC SULFUR	ETHEREAL SULFUR	TOTAL PROTEIN	ALBUMIN	GLOBULIN	A./G.
				10 MIN.	18 MIN.						
1	35	1.54	5.6	63.6	45.4						
2	40	1.17	3.7	62.5	50.0						
4	31	1.12	3.6	35	25						
13	30	0.98	4.8	56	40	3.6	0.6				
14	45	1.34	3.0	66.6	55.5	2.9	0.8				
6	55	2.08	5.6					8.41	4.63	3.73	1.22
9	68	2.80	8.7					5.5	3.76	1.74	2.16
10	28	1.28	3.2					6.27	4.15	1.18	1.95

*Normal values: 10 min., 55 per cent or less, 18 min., 35 per cent or less.

Table III shows the results of 5 Rose Bengal liver function tests, 3 total protein and albumin and globulin determinations, and 2 sulfur determinations.

1. The liver function tests showed dye retention in 4 of 5 determinations.

2. The blood protein determinations done on 3 patients were within normal values as was the distribution between the albumin and globulin fractions.

3. Inorganic and ethereal sulfates of the blood were found normal in 2 patients.

TABLE IV

CASE NO.	HB.	R.B.C.	W.B.C.	B.M.R.	ICTERIC INDEX	TEMPERATURE
1	35	1.54	5.6	plus 28	18	99
2	40	1.12	3.7	plus 13	15.2	98.8
3	40	1.48	4.8	plus 30	16	99
4	31	1.12	3.6	plus 21	7	98.8
5	58	2.35	3.6	0	20	98.2
6	55	2.08	5.8	plus 17	15	99.4
7	55	1.62	5.4	plus 6	7.1	98.4
8	56	2.32	5.6	plus 12	8	98.2
9	68	2.80	8.7	plus 15	5.7	98.2
10	28	1.28	3.2	plus 22	21.4	99.4
11	40	1.20	4.3	plus 9	22	99.2
12	40	1.80	3.8	plus 18	19	98.6
13	30	0.90	4.8	plus 32	33	98.6
14	45	1.34	3	plus 28	18	99.2
15	54	1.85	3.8	plus 20	13.3	98.2

Table IV shows the basal metabolic rates of 15 patients and their icteric indices. The temperatures are included merely to indicate that at the time the basal metabolic rates were taken patients had no significant temperature.

1. Three of the 15 basal metabolic rate determinations only were normal, the other 12 being definitely elevated.

2. The icteric index determinations were normal in 4 of 15 patients on whom they were done.

SUMMARY

1. Total lipoids on all determinations were increased much above normal values. The fatty acids were very much diminished in all the determinations, as were the "lecithins" and the lipoid phosphorus. Cholesterol seemed normally distributed between the free and esterified forms, and showed values that were normal or slightly diminished. Only one cholesterol determination was below 100 mg.

2. One of 6 amino acid determinations showed a marked increase, while the other 5 were within normal values.

3. Calcium, phosphorus, uric acid, creatinine, sugar, chlorides, and carbon dioxide determinations were found normal. One nonprotein nitrogen and one urea nitrogen determination showed an increase above normal.

4. Total protein, albumin, and globulin values on 3 patients were normal.

5. Inorganic sulfates and ethereal sulfates on 2 patients were normal.

6. Four Rose Bengal liver function tests showed dye retention, and the fifth was normal.

7. The icteric index was increased in 73.3 per cent of the cases.

8. The basal metabolic rate was increased in 80 per cent of the cases.

9. One glutathione determination done on a patient who had hemoglobin 16 per cent and a red blood cell count of 0.710 showed 14.5 mg. per cent of reduced glutathione and none of the oxidized. The patient died two days later a section of liver taken from the autopsy and extracted with saline showed 168.7 mg. of reduced glutathione per 100 Gm. of liver. No oxidized glutathione was demonstrated in the saline liver extract.

NOTE.—Blood lipid determinations were all done by the Bloor method and were begun before the work of Christensen¹⁰ and Van Slyke and Folch¹¹ was published. This study of the lipoids in pernicious anemia is being continued in our laboratories by both the Bloor method and the modifications suggested by the authors mentioned.

REFERENCES

1. Peters, J. P., and Van Slyke, D. D.: *Quantitative Clinical Chemistry*, Baltimore, 1931, Williams & Wilkins Co., Vol. I.
2. Mathews, A. P.: *Principles of Biochemistry*, Baltimore, 1937, William Wood & Co.
3. Casele, W. R., and Minot, G.: *Pathological, Physiological and Clinical Description of the Anemias*, New York, 1936, Oxford University Press.
4. Naegeli, O.: *Blutkrankheiten und Blutdiagnostik*, ed. 5, Berlin, 1931, Julius Springer.
5. Clough, P. W.: *Diseases of the Blood*, New York, 1929, Harper & Brothers.
6. Bloor, W. R.: Total Lipoids, *J. Biol. Chem.* 17: 377, 1914.
7. Bloor, W. R.: Fatty Acids, *J. Biol. Chem.* 77: 53, 1928.
8. Bloor, W. R., Pelkam, K. F., and Allen, D. M.: Total Cholesterol, *J. Biol. Chem.* 52: 191, 1922.
9. Peters, J. P., and Van Slyke, D. D.: *Quantitative Clinical Chemistry*, Extraction of Lipoids, p. 884; Determination of Phosphorus, p. 882, Baltimore, 1932, Williams & Wilkins Co., Vol. II.
10. Christensen, H. N.: Contaminants of Blood Phospholipids, *J. Biol. Chem.* 129: 531, 1939.
11. Van Slyke, D. D., and Folch, J.: Nitrogenous Contaminants of Petroleum Ether Extracts *J. Biol. Chem.* 129: 539, 1939.

LABORATORY METHODS

GENERAL

A SIMPLE NONTRAUMATIZING METHOD OF INDUCING PNEUMOCOCCIC INFECTION IN ALBINO RATS*

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WITHIN the past few years there have been developed new therapeutic agents for use in the treatment of pneumonia. Since there is great variability in the severity of human pneumococcic infections from year to year, it is necessary before these substances are used clinically to determine first their efficacy and toxicity under controlled conditions.

Tests in vitro partially demonstrate the efficacy, but obviously give no clue as to the toxicity of these preparations. To determine their toxicity and complete effectiveness in infection, experimental animals must be used.

It is well known that albino rats can be infected with pneumococci. Intraperitoneal inoculation and intrabronchial instillation of pneumococci are two procedures which have been used to infect them. The former causes a high incidence of infection and the dose of organisms can, with reasonable accuracy, be controlled. This method, however, does not reveal the effectiveness of therapy when there is pulmonary involvement.

With the latter method, originated by Nungester and Jourdonais,^{1,2} and used by Gross and Cooper,^{3,4} in the study of antipneumococcic drugs, in which pneumococci are suspended in gastric mucus, and instilled into a bronchus, infection is irregular and the recovery rate of the untreated or control animals is too high to warrant accurate evaluation of the therapeutic agent. Even though carefully performed, this procedure causes trauma which interferes with the interpretation of therapeutic efficacy and incidentally the pathogenesis.

A method, therefore, is required which, with a minimum of trauma, will produce a high incidence of pneumococcic infection with pulmonary involvement, and a constantly high fatality rate in infected animals. With these facts in mind, the following procedure is suggested.

METHOD

An unanesthetized rat, weighing about 300 Gm., is fastened with rawhide thongs to a small animal board (Fig. 1) designed for this procedure. A wire loop, attached to the undersurface of the board with a rubber band, is slipped

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over the animal's upper incisor teeth, and a strip of gauze is placed over the lower incisor teeth to pull the jaw open.

A pneumococcus culture, consisting of the washings of the peritoneal cavity of a white mouse infected with pneumococci,* is suspended in 15 c.c. of a physiologic solution of sodium chloride. This is sprayed into the pharynx and nares of the rat by means of an atomizer, the bulb being squeezed four times. This culture contains, as a result of the peritoneal inflammation, natural mucin in addition to the other components of an inflammatory exudate.

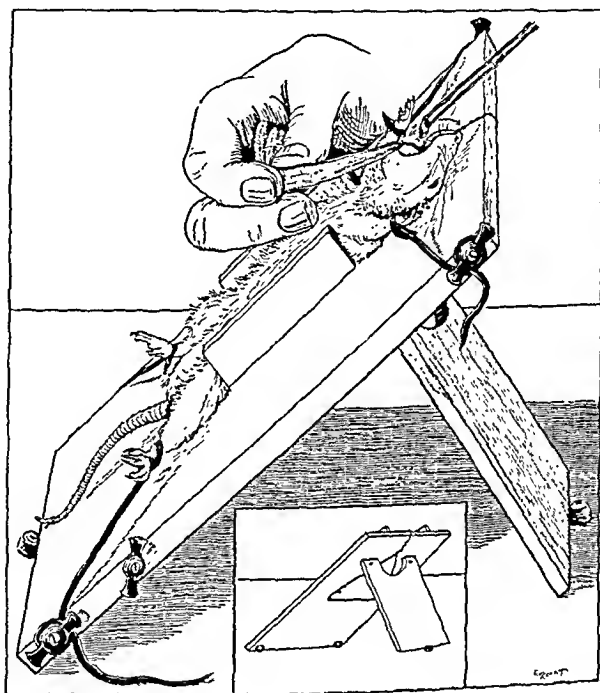


Fig. 1.

After preliminary treatment with soap and water, followed by acetone and alcohol, blood for culture is obtained from the tail of the animal by snipping off the tip with a pair of sterilized seissors. The drops of blood appearing at the cut end are placed directly on a blood agar plate and then streaked over the surface with a wire loop.

RESULTS

Of 131 animals thus sprayed 107, or 81.6 per cent, became infected as manifested by positive blood cultures.

Only those animals in which a growth of pneumococci in the blood culture was obtained were considered as having been infected. These animals invariably died. Although positive cultures have been obtained as early as four hours, the majority of the animals developed positive cultures between six and twenty-four hours after spraying.

*RI strain pneumococci were used in all experiments.

In the animals that died of pneumococcal infection, patches of pneumonia, sometimes involving an entire lobe or multiple lobes, were noted. In the majority of these animals this patchy pneumonic process was associated with empyema and purulent pericarditis. A purulent peritonitis sometimes developed and, in a few, meningitis occurred.

When the rats died of any of the above conditions a growth of pneumococci was obtained from the heart's blood.

DISCUSSION

The method of infecting rats with pneumococci in which pulmonic infection and bacteremia were common is nontraumatic, easily and rapidly performed, and permits, when necessary, large numbers of animals to be used for therapeutic studies.

Many investigators have sprayed the upper respiratory tract of animals attempting to produce pneumococcal pulmonary infections, but the results were generally unsatisfactory. In these cases the pneumococci used were grown in artificial media. Pneumococcal pneumonia in man is most frequently caused by pneumococci which have recently been in contact with a human host or carrier. It is known that organisms recently in contact with an animal host are more virulent than those grown in artificial media from eighteen to twenty-four hours. In order to simulate as closely as possible the conditions in human infection, when attempting to infect rats, we have used pneumococci in mouse peritoneal exudate, containing natural mucin, because this procedure appears to satisfy this requirement best. Not only are these organisms more virulent, but, as pointed out by Nungester and Jourdonais,² gastric mucin, when inoculated intrabronchially with pneumococci, hinders or prevents rapid phagocytosis of these organisms by the leucocytes of the infected animals. Such conditions probably exist in human infection.

SUMMARY

1. A simple nontraumatizing method of infecting rats with pneumococci has been described, in which pneumococcal exudate from the peritoneal cavity of the mouse is used because of the increased virulence of pneumococci recently in contact with an animal host and because of the natural mucin content of the exudate.
2. A high incidence of infection, 81.6 per cent, was produced as manifested by positive blood cultures, and at autopsy, by pneumonia, empyema, purulent pericarditis, peritonitis, or meningitis.

We wish to express our appreciation to Doctors Tasker Howard, J. Hamilton Crawford, Wade W. Oliver, and Arnold Eggerth for their aid and criticism in the preparation of this paper.

REFERENCES

1. Jourdonais, L. F., and Nungester, W. J.: Intratracheal Inoculations in the Rat, *Science* 81: 74, 1935.
2. Nungester, W. J., and Jourdonais, L. F.: Mucin as Aid in Experimental Production of Lobar Pneumonia, *J. Infect. Dis.* 59: 258, 1936.
3. Gross, P., and Cooper, F. B.: Efficacy of p-Aminobenzenesulfonamide in Experimental Type III Pneumococcus Pneumonia of Rats, *Proc. Soc. Exper. Biol. & Med.* 36: 225, 1937.
4. Gross, P., and Cooper, F. B.: p-Aminobenzenesulfonamide and Antipneumococcal Serum Therapy in Type I Pneumococcal Infections of Rats, *Proc. Soc. Exper. Biol. & Med.* 36: 535, 1937.

ORAL AND NASAL SIMULTANEOUS ASPIRATORS AND THEIR USE IN GASTROENTEROLOGY

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THE duodenum does not play a very important part in the physiology of digestion, except that it receives, aside from its own secretion, the combined secretions of the pancreas, liver, and biliary tract. It is here that the material coming from the stomach meets these various secretions. Thus the duodenum serves not only as a melting pot, but also as a conduit for conveying the combined material into the jejunum and ileum where the final process of digestion occurs.

Due to its position investigators made numerous attempts in the past to intubate the duodenum, among the first of whom were Hemmeter and Kuhn. The instruments they employed were crude and impractical and were therefore abandoned.

Max Einhorn experimented for a number of years with various tips and buckets, and finally, in 1909, developed the first practical apparatus which he called the duodenal pump.¹ Since that time eminent gastroenterologists have developed various types of tips, each claiming particular advantages, but all alike in principle, differing only in their size, weight, and number of perforations (Fig. 1).

In 1938 I developed a bucketless tube,² a radical departure from the other tubes in use. The apparatus is 50 inches in length (Fig. 2), 14 French in diameter, and its terminal end encases an elongated lead dropper, narrow and tapered above, but increasing in size below. Two openings, slotted in appearance, are situated just above the dropper, $\frac{1}{2}$ inch apart, on opposite sides of the tube.

The advent of the duodenal tube enabled physiologists, clinicians, and research workers to make a comprehensive survey and study of the functions of the stomach, liver, and pancreas. Yet, in their research work, they still felt the growing need for an instrument which would intubate both the stomach and duodenum, and permit a simultaneous withdrawal of their respective contents.

Max Einhorn³ in 1918 demonstrated an apparatus (Fig. 3A) by means of which the gastric and duodenal contents could be obtained at the same time. His aspirator consisted of a long soft rubber tube, about 12 French in diameter, divided into two separate canals, one leading to a terminal perforated capsule and the other to a perforated hollow metal connector situated about 25 cm. from the end of the tube. The proximal end of the tube branched off into two side tubes each provided with stopcocks, one marked *D* for the duodenum and the other *S* for stomach.

This double aspirator was provided with markings starting from the terminal end of the tube, and was introduced in the same manner as the usual duodenal tube, so that while the bucket entered the duodenum, the perforated connector lay within the limits of the stomach, thereby allowing the duodenal contents to be aspirated separately through the terminal tip and the gastric contents through the perforated connector.

For the simultaneous sampling of the gastroduodenal contents, Rehffuss first employed a method in which he used two separate tubes of different lengths. The patient first swallowed the longer tube in the usual way, and then, after it was ascertained that the tip had entered the duodenum, he swallowed the shorter tube.






					
	ALLPORT	LAX LITHON	REHFFUS	LYON	ROUSSEAU
LENGTH	INCHES. d/8.	7/8	8/8	9/8.	10/8
WEIGHT	GRAMS. 104.	59	63	72	170
DIAETER	FR. CH. 31.	23	29.	27	27.

Fig. 1.—Various tips used in gastrointestinal work.

To simplify it further, Rehffuss employed a second method⁴ whereby he attached the capsule of the shorter tube to the wall of the longer tube (Fig. 3B) at a distance of about 25 cm. from its end, so that when the distal tip was in the duodenum, the proximal capsule lay within the limits of the stomach.

In 1927 Kunstler, impressed by the findings of Garbat⁵ and Carlson⁶ that duodenal feeding promotes a rise of gastric acidity, suggested that constant and complete neutralization of gastric acidity in duodenal feedings could be accomplished by administering an alkaline solution by means of the continuous drip method. He designed for that purpose a special double tube.⁷ This consisted of an ordinary tube (Fig. 3C) to whose wall was vulcanized at a point 20 cm. from its bucket a shorter tube, smaller in caliber and open at its end for a distance of 70 cm., thus leaving the remaining 20 cm. free. His idea of treatment was to administer two-hour duodenal feedings through the longer tube and introduced a weakly alkaline solution for the full twenty-four hours by the drip method through the shorter tube.

The well-known Bayliss and Starling⁸ discovered the substance secretin which is formed when the acid contents of the stomach pass into the duodenum, which in turn, stimulates the production of pancreatic secretion and its enzymes.

This work has been confirmed in its major aspects by most physiologists and, hence, secretin has been the substance used when a substantial increase in the volume of the pancreatic secretion was desired.

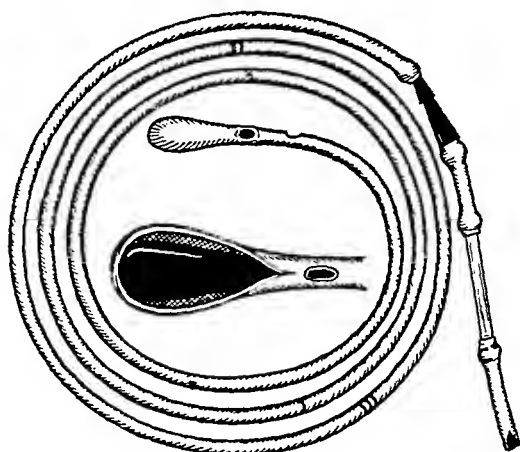


Fig. 2.—Einhorn's bucketless lead weighted gastroduodenal tube.

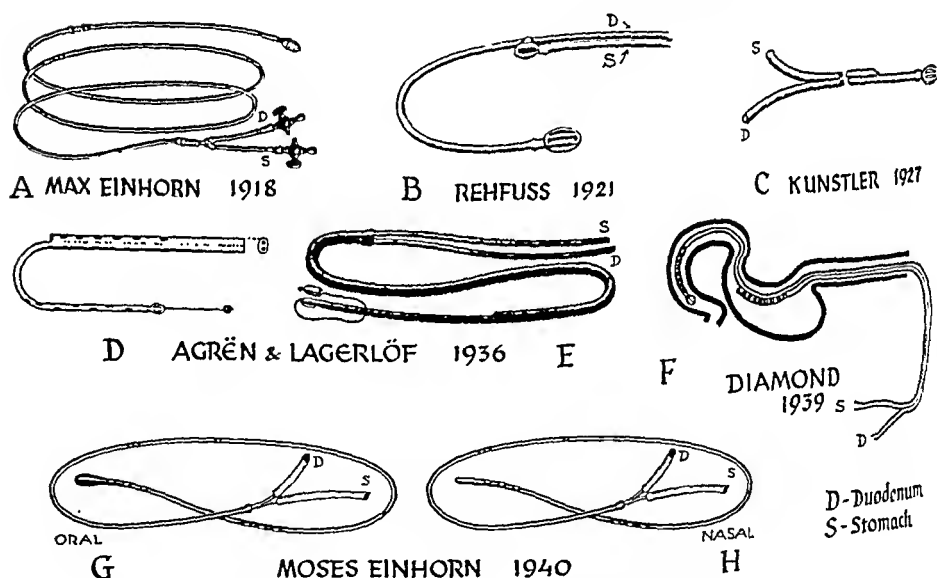


Fig. 3.—Various simultaneous gastroduodenal aspirators.

The Swedish investigators, Ågren and Lagerlöf,⁹ made an extensive study of pancreatic secretion in man after intravenous administration of secretin and designed a special double tube for that purpose (Fig. 3D, E). Their tube consisted of two tubes free for the first 20 cm. and fused together for a distance of 70 cm. The duodenal side, which extended for a distance of 20 cm. and had a capsule at its end, featured seven oval-shaped openings on its last 8 to 10 cm. of tubing. The gastric tube had five to six openings on its side. The whole of this 70 cm. of fused tubing was encased in a thin rubber sheath to facilitate swallowing. In order to accelerate the entrance of the duodenal tube into the duodenum, they tied to its end a fine silk cord, 10 cm. in length,

to which was attached a lead ball 8 mm. in diameter. The tube was swallowed in the usual way, and when the tip entered the duodenum, the openings of the gastric tube remained in the stomach area.

In this country, Diamond¹⁰ recently made extensive studies on the use of secretin as a clinical test of pancreatic function and used a specially designed gastroduodenal tube. His apparatus permitted the separate collection of gastric and duodenal contents by preventing the admixture of the two secretions, thus assuring the collection of pure duodenal juice (Fig 3*F*).

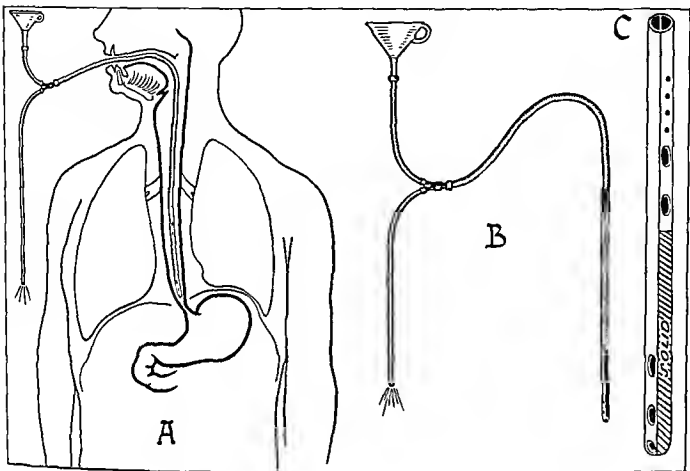


Fig. 4.—Einhorn's double channel esophageal lavage tube.

In my article on "The Treatment of Cardiospasm,"¹¹ I described a double lumen tube (Fig. 4) for esophageal lavage. Its basic principle is the same as that which has for some time been used in colonic irrigation and which has subsequently been adopted by Miller and Abbott for intestinal intubation. The tube is 28 French in diameter, 30 inches in length, and is bisected into two distinct canals. One of the canals terminates at a point four inches short of the tip, while the remainder of its lumen is filled with solid rubber. Immediately above this solid portion the walls feature a few wide openings. The second canal extends to the very end of the tube and has at its terminal end a few oval perforations.

The idea occurred to me that by combining the main features incorporated in the bucketless and double lumen esophageal tube, I would have a very simple and efficient either oral or nasal simultaneous gastroduodenal aspirator (Fig. 3*G*, II).

The oral tube* (Fig. 5*F*) is 50 inches in length, 16 and 18 French in diameter, semisoft in consistency, flexible, and of good resiliency. Its lumen

*Made by Clay-Adams Co.

is equally divided into two channels (Fig. 5A), semilunar in shape. Its terminal end, 1 inch in length, encases an elongated drop-shaped lead sinker (Fig. 5Ga), narrow and tapered above, but increasing in size at the bottom, this portion including the sinker with its layer of rubber being 150 grains in weight, 1 inch in length, and 27 French in diameter at the bottom, the top being of the same size as the tube. The duodenal side of the double channel extends down to the top of the sinker and has three oval openings slotted in appearance and $\frac{1}{2}$ inch apart. The small space between the sinker and the lower opening is filled with rubber. The gastric side of this double channel ends at a point about 7 inches above the sinker; the remainder of its lumen is filled with lead shot (Fig. 5Gb). Immediately above the lead-filled portion, there are three openings, oval in shape and $\frac{1}{2}$ inch apart.

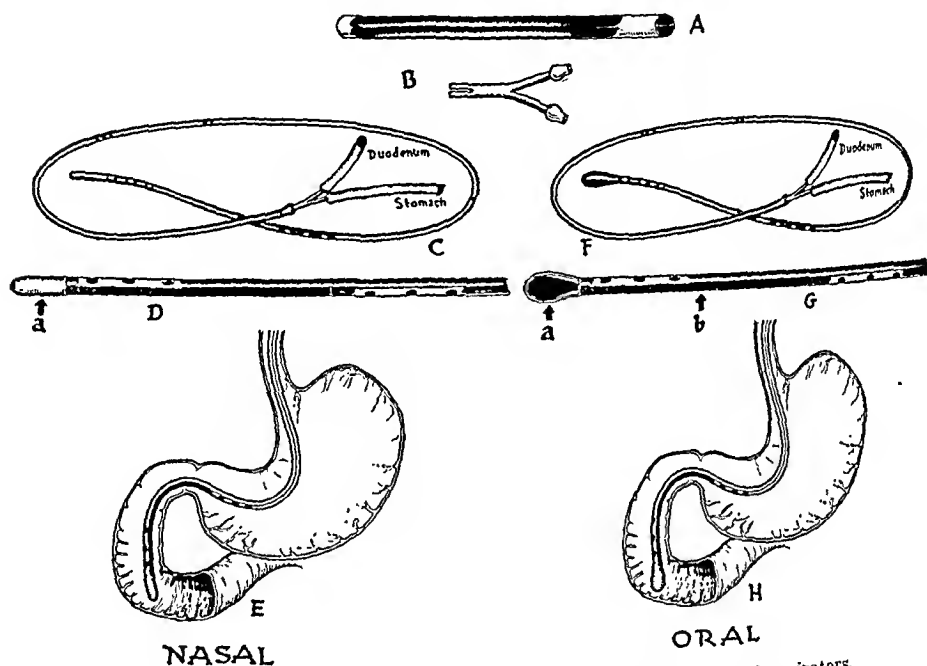


Fig. 5.—Einhorn's nasal and oral simultaneous gastroduodenal aspirators.

The nasal tube (Fig. 5C) is designed in the same manner as the oral, except that it is 16 French in diameter and terminates in a catheter-like tip (Fig. 5Da), which is made of solid impregnated lead rubber for $2\frac{1}{2}$ inches.

For the oral tube the markings are as follows: the one-ring mark is placed 17 inches from the end of the tube, and represents the distance from the lips to the cardia; the two-ring mark, at 28 inches, represents the distance from the lips to the pylorus; and the three-ring mark, at 33 inches, permits 5 inches of the terminal end of the tube to enter the duodenum (Fig. 5H) and leaves a space of 3 inches from the lower opening of the gastric channel.

In the case of the nasal tube the one-ring mark is placed at 19 inches, the two-ring mark at 30 inches, and the three-ring mark at $36\frac{1}{2}$ inches. It is understood, however, that the physician's judgment should always determine the amount of tubing for each individual and he should not rely entirely on the markings.

The special double channel connector (Fig. 5B) consists of two narrow metal tubes soldered together in the middle, branching off distally into two distinct separate canals, each of which is cemented into its proximal opening of the tube to render it airtight. Proximally, the connector bifurcates into two channels, one representing the gastric outlet and the other the duodenal.

ADVANTAGES

Oral Tube.—1. The tube is simple in construction and no fusion of tubes is necessary, thereby facilitating swallowing.

2. The fear the patient usually experiences that the metal bucket may become detached is eliminated, as the lead dropper is encased in the tube.

3. Swallowing of the tube is accelerated because of the weight of the dropper, which is sufficient to permit its passage through the esophagus with no discomfort to the patient.

4. The tube maintains its intended course along the stomach pathway because of the lead-shot compartment and the weight and proper arrangement of the lead sinker, which keeps it in its proper place in its progress to the pyloric opening.

5. Due to the special distribution of the weight of the sinker, which anchors the tube securely in the duodenum, there is no likelihood of regurgitation of the bucket from the duodenum into the stomach, as is usually the case with other tips.

6. No discomfort is encountered at the glottis upon removal of the tube, because of its tapered shape and gradual decrease in size at the neck.

7. There is no need for silver- or gold-plating the metal bucket to avoid impairment of the mucous membrane, since the sinker is encased in rubber.

8. The openings of the tube have rubber margins, thereby eliminating the possibility of cutting the mucous membrane upon suction, as is usually the case with metal buckets.

Nasal Tube.—1. The $2\frac{1}{2}$ inch solid rubber catheter-shaped tip at the terminal end facilitates the introduction and passage of the tube through the nares.

2. The weight of the lead-impregnated rubber tip and the lead-shot compartment facilitates the passage of the tube through the esophagus, maintains it in its proper position along the stomach pathway, and aids its entrance into the duodenum.

3. There is less possibility of the lower part of the tube regurgitating into the stomach as the weight of the solid lead-impregnated rubber portion and the lead-shot compartment keeps it securely in the duodenum.

TECHNIQUE

Oral Intubation.—The patient reports on an empty stomach and is placed in an upright position on a chair or in bed, and is instructed to protrude his tongue to form a groove. The operator seizes the terminal lead sinker, previously moistened, between the thumb and forefinger, and places it on the grooved portion of the tongue. The patient is instructed to open his mouth wide and say "Ah." The operator then inserts the sinker far enough into the

mouth under the uvula and instructs him to close his mouth and concentrate on the act of swallowing. The tube, due to its lead weight, will be carried down slowly through the esophagus.

As soon as the tube has reached the first-ring mark, the patient is placed in a reclining position on the right side, with the right leg and knee stretched straight and the left leg and knee flexed, overlapping the right.

The patient is instructed to swallow the tube very slowly, an inch at a time, over a period of twenty to twenty-five minutes, until the third-ring mark is reached, so that the terminal end will enter the duodenum.

After the patient has swallowed a few inches of the tubing while lying on the right side, it is a good practice to inject a small amount of lukewarm water through the gastric canal in order to relax the pyloric opening and stimulate the peristaltic action of the stomach, thereby facilitating and accelerating the entrance of the terminal portion of the tube into the duodenum.

Upon completion of the examination, the operator withdraws the first seven inches of the tube while the patient is in a recumbent position, and the balance of the tube while the patient is in the upright position.

In case no bile is obtained, the operator should withdraw the tube up to a point midway between the first- and second-ring marks and then instruct the patient to swallow it again.

Nasal Intubation.—The terminal end of the tube, which has previously been dipped in glycerin, is introduced through the nose for a distance of six inches, and the patient is then asked to simulate the act of swallowing, while the operator pushes the tube which will be carried down slowly in the esophagus because of its lead-weight compartment.

As soon as the first-ring mark is reached, the patient is turned on the right side and the same procedure is followed as in oral intubation.

Tests for Determining the Entrance of the Terminal Tip Into the Duodenum.—1. It is possible to determine the entrance of the tip into the duodenum with the aid of fluoroscopic and x-ray examinations. Although this method is undoubtedly reliable, I believe that it should not be used in routine practice, since in the course of removal of the patient to the x-ray room and of manipulation, the terminal portion of the tube may easily slip back into the stomach even with the lead-weighted tip.

2. By observing the characteristic differences in color, viscosity, and reaction of both specimens obtained from the two outlets. The duodenal specimen will be viscid, golden yellow in color, and negative to Congo red, whereas the gastric specimen will be clearer, perhaps bile-tinged, but positive to Congo red.

3. If, after injecting a mixture of barium or any other colored solution into the duodenal outlet, no return is obtained on the gastric side, the terminal tip has entered the duodenum.

USES

Medical.—1. For obtaining a simultaneous specimen from the stomach and duodenum.

2. For determining the effect on the duodenal contents of substances introduced into the stomach and vice versa.

3. In peptic ulcer cases where duodenal feeding and intermittent or continuous neutralization by drip method is desired.
 4. In cases of gastric atony, nervous dyspepsias, or any form of vomiting where duodenal gavage and gastric drainage are indicated.
 5. In biliary drainage.
 6. In cases where esophageal or gastric continuous lavage is required.
- Surgical*.—1. In preoperative and postoperative gastrointestinal and abdominal surgery, where simultaneous and continuous gastrointestinal drainage¹² is wanted.
2. In cases where tubal feeding and drainage are required.
 3. In cases of gastroenterostomy.

I wish to express my gratitude to Mr. L. T. Hillborn, president of the Clay-Adams Co., for his kind cooperation in the development of the tubes

REFERENCES

1. Einhorn, Max: A New Method of Catheterizing the Pylorus and Duodenum, New York Med. Rec. 76: No. 15, 1909.
2. Einhorn, Moses: New Bucketless Lead Weighted Gastro-Duodenal Tube With a Review of the American Contribution to the Development of These Tubes, Am. J. Digest. Dis. 5: 77, 1939.
3. Einhorn, Max: The Duodenal Tube, Philadelphia, 1926, F. A. Davis Co.
4. Rehfuess, M.: Diagnosis and Treatment of Diseases of the Stomach, Philadelphia and London, 1927, W. B. Saunders Co., pp. 103, 122.
5. Garbat, A. L.: Gastric Secretion in Response to Duodenal Feeding, Arch. Int. Med. 32: 771, 1928.
6. Carlson, A. J.: Secretion of Gastric Juice in Health and Disease, Physiol. Rev. 3: 1923.
7. Kunstler, M. B.: A New Duodenal Tube for the Treatment of Peptic Ulcer, M. J. & Rec. 125: 466, 1927.
8. Bayliss, W. M., and Starling, E. H.: J. Physiol. 24: 99, 1899.
9. Agren, G., and Lagerlöf, H.: Pancreatic Secretion in Man After Intravenous Administration of Secretin, Acta. med. Scandinav. 90: 1, 1936.
10. Diamond, J., Siegel, S., Gall, M., and Karlen, S.: The Use of Secretin as a Clinical Test of Pancreatic Function, Am. J. Digest. Dis. 6: 366, 1939.
11. Einhorn, Moses: The Treatment of Cardiospasm Illustrating a New Cardiospasm Dilator and a Bisected Esophageal Tube, Rev. Gastroenterol. 6: 499, 1939.
12. Einhorn, Moses: Nasal Simultaneous Gastroduodenal Aspirator: Its Use in Postoperative Gastrointestinal and Abdominal Surgery, Surg., Gynec. & Obst. 72: 48, 1941.

A STUDENT SPHYGMOSCOPE*

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THE desirability of providing the student with laboratory experience with the type of sphygmomanometer devised by Erlanger led us some years ago to modify the sphygmoscope developed by Erlanger and Meek¹ so that advantage could be taken of apparatus available in the student equipment.

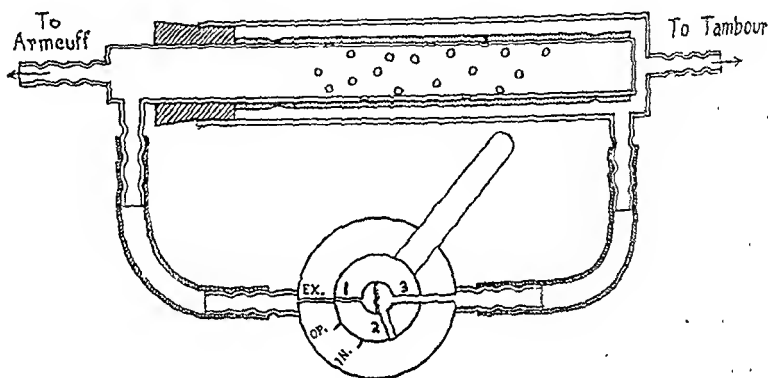


Fig. 1.—The student sphygmoscope.

The sphygmoscope shown in Fig. 1 has been in use for twelve years with a minimum of servicing by the mechanic. It is essentially the same as that of Erlanger and Meek, excepting for the arrangement of the control valve. The parts are metal. Brass tubing is used instead of glass. The rubber membrane surrounding the inner tube may be quickly made from sheet rubber of suitable thickness by cementing overlapping edges. The control valve replaces the compressive compound stopcock in the Erlanger sphygmomanometer. A capillary glass or metal stopcock placed on a side arm in the connection to the tambour provides a leak which may be easily adjusted so that it protects the tambour against slow large changes in the volume of the outer chamber of the sphygmoscope, but does not interfere with the recording of the oscillations; or a similar needle point valve may be soldered on the cup of the tambour to serve the same purpose. The sphygmoscope and control valve are mounted on a board. On all dimensions are 8 by 4½ by 2½ inches, permitting compact storage.

Operation of Control Valve.—Three positions are available:

(1) Exhaust position (shown). Arm cuff and outer chamber of sphygmoscope are in connection with the outside through exits 1 and 3. A baffle plate in the central outlet of the cock prevents the gust of air from the arm cuff from disturbing the tambour. This position is used for deflating the arm cuff when using the intermittent escapement method. The diameter of exit 1

*From the Department of Physiology, St. Louis University School of Medicine.
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85 mm.

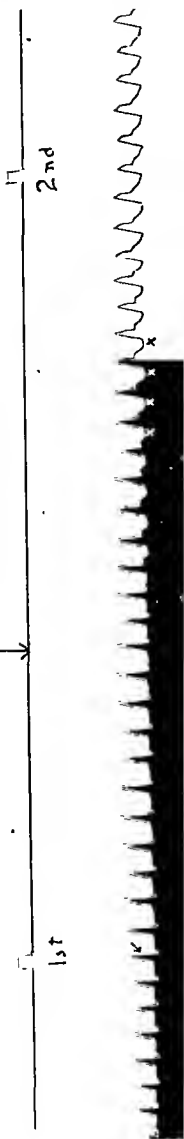


Fig. 2.—Illustrative record made with student equipment. Lower record sphygmoscope oscillations. Note flattening in diastolic portion of curve at X, and its disappearance when diastolic pressure (auscultatory) is reached. Upper record: arm cuff pressure. Middle record: auscultatory systolic and diastolic pressures at first and second signals.

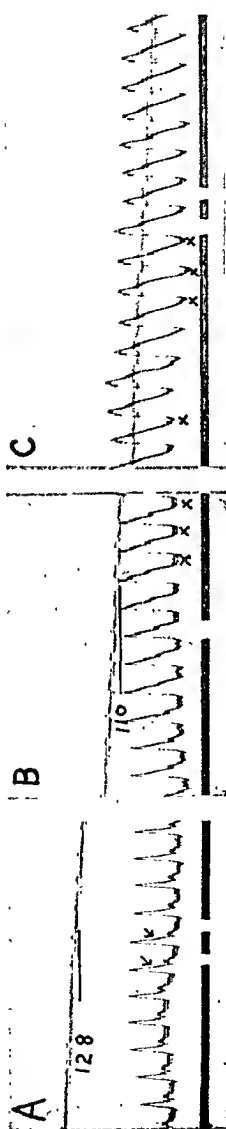


Fig. 3.—Sections from optical record. A, Auscultatory systolic pressure (double signal) at 128 mm. Hg. B, Cuff pressure 110 mm. Hg. C, Auscultatory diastolic pressure (double signal) at 93 mm. Hg.

made small enough so that the deflation is readily controlled. (2) Intake position. Exit 1 is in line with the scratch mark IN. Exit 2 is in connection with the outer chamber of the sphygmoscope and so prevents the large volume changes here during inflation of the arm cuff from affecting the tambour. (3) Operating position. Exit 1 is in line with the scratch mark OP. All exits are closed in this position. The oscillations of the sphygmoscope are now transmitted to the tambour. This position is used when the method of continuous escapement is employed, the tambour leak being adjusted in proportion to the rate of fall in arm cuff pressure which is controlled by the air escape valve on the cuff. The method of intermittent escapement may also be used with this position. This position is also used when one wishes to record continuously variations in arterial pressure. The sphygmoscope is very useful for student experiments of the latter type.

Criteria of Systolic and Diastolic Pressures With Sphygmoscope.—These can be studied by the student by arranging to record arm cuff pressures with a recording manometer, and signaling auscultatory systolic and diastolic criteria while the sphygmoscope oscillations are being recorded. A typical performance using a Marey tambour (Harvard Apparatus Co.) is illustrated in Fig. 3. The systolic sign, as pointed out by Erlanger, seems to be "the first clear increase in the spread of the limbs at the base of the pulse wave." The diastolic criteria of Erlanger are less apparent. Many records have shown that a flattening in the catacrotic portion of the pulse curve disappears at pressures very near those at which the Korotkow sounds become dull and muffled. This is more clearly shown in the optical records of Fig. 3. The wave is completely supported throughout the pulse cycle at lower pressures. This relationship is not entirely regular. The flattening may disappear either slightly above or slightly below the pressure at which muffling occurs.

REFERENCE

1. Erlanger, J., and Meek, W. J.: Adjustable Sphygmoscope for Recording Sphygmomanometer, J. LAB. & CLIN. MED. 12: 172, 1926.

DEXTROSE YEAST EXTRACT MEDIUM FOR THE ISOLATION OF FUNGI FROM SPUTUM*

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RAY BROOK, N. Y.

SABOURAUD'S medium with a pH of 5.5 is in general use for the isolation of fungi from the sputum; however, it inhibits only to a limited degree the growth of bacteria commonly found in the sputum when incubated at a temperature of 37° C. One or more transplants may be required before the fungus is finally recovered in pure culture. The growth of the bacteria may be further inhibited by gently swabbing the surface of the medium with a tenth-normal solution of hydrochloric acid previous to the implantation of the sputum. The application of the acid to the medium does not interfere with the growth of fungi. The foregoing observation suggested the advisability of materially increasing the acidity of Sabouraud's medium for the recovery of fungi from the sputum.

A series of experiments disclosed that the optimal pH for the growth of fungi and the prevention of bacterial growth is 4.0. In order to bring Sabouraud's medium to this pH, it is necessary to modify in part the standard method of preparation as follows:

Dissolve the peptone and agar in distilled water by heating in the autoclave for fifteen minutes at 15 pounds pressure. Add a sufficient amount of an aqueous solution of 20 per cent dextrose to bring the dextrose content of the medium to 4 per cent. Under sterile conditions, and while the medium is still in the liquid state, adjust to the desired pH. The medium can be poured immediately or allowed to solidify in the container for future use.

The adjustment of Sabouraud's medium to a pH of 4.0 in the manner here described requires detailed attention and considerable time. Moreover, Sabouraud's medium is not kept in stock in many laboratories and consequently is not always available to the physician. These objections are overcome by the following simple liquid medium:

Dextrose	8.0 Gm.
Sodium chloride	1.7 Gm.
Yeast extract (Difco)	0.5 Gm.
Distilled water to make	200 c.c.
Adjust to pH 4.0	

The above medium should not be diluted when determining the pH, since it is buffered to only a slight extent if at all, and the addition of water would decrease the hydrogen-ion content.

The experiments here outlined indicate the relative value of Sabouraud's medium with pH values of 5.5 and 4.0, and dextrose-yeast-extract medium with

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a pH of 4.0 in the recovery of fungi from the sputum. In a few instances fungi present in the sputum, for example, the *Penicillium*, grow better at room temperature than at 37° C. It is advisable, therefore, as a routine procedure, to grow the cultures at both these temperatures.

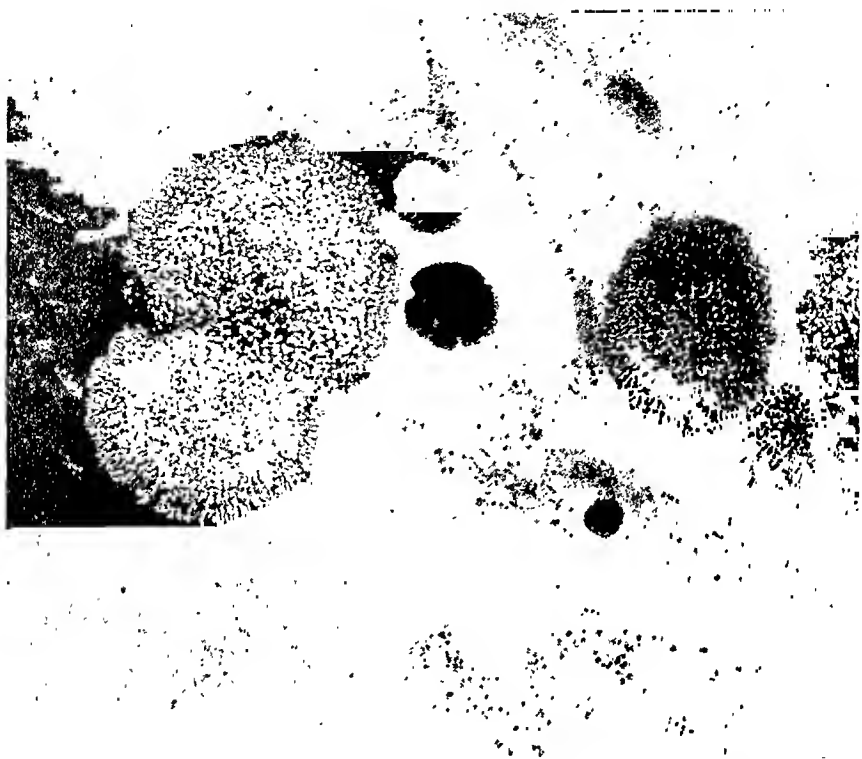


Fig. 1.—Fungus and bacterial colonies on Sabouraud's plate with pH 5.5.

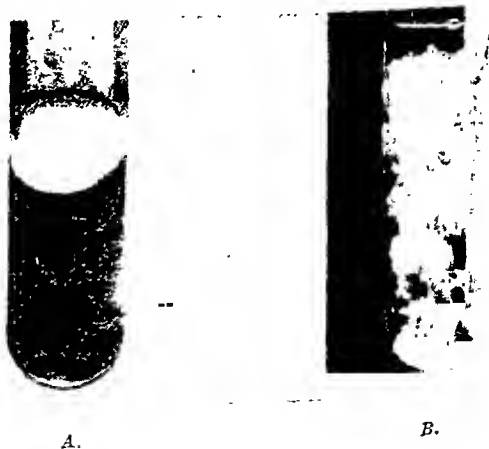


Fig. 2.—Pure culture in liquid media with pH 4.0. A, *Aspergillus fumigatus*; B, *Coccidioides immitis*.

Fifty specimens of tuberculous sputa were collected in sterile Petri dishes. A purulent portion from each specimen of sputum was implanted on these three media. The implanted medium was incubated at 37° C. and observed

daily for a period of ten days. Sixteen of the fifty specimens of sputa yielded cultures of *Monilia* on each of the three media. In no instance on Sabouraud's medium with a pH of 5.5 were the colonies of *Monilia* found in pure culture; but numerous colonies of bacteria were present on all fifty plates. The number of bacterial colonies was greatly reduced on Sabouraud's medium with a pH of 4.0, and nine of the sixteen specimens gave pure cultures of *Monilia*. Sputum implanted in fifty tubes of dextrose-yeast-extract medium gave no bacterial growth as determined by cultures on nutrient agar plates, and the *Monilia* was recovered in pure culture from sixteen tubes.

Cultures of various fungi (*Coccidioides immitis*, *Aspergillus fumigatus*, *Aspergillus niger*, *Oidium*, *Monilia albicans*, *Geotrichum*, *Cryptococcus*, *Torula*, *Streptothrix*, *Actinomyces*, and *Sporotrichum*) were intimately mixed with sputa from tuberculous patients and implanted on Sabouraud's medium with pH values of 5.5 and 4.0, and on dextrose-yeast-extract medium with a pH of 4.0. Colonies of each of the above-mentioned fungi were found on all three media. However, many bacterial colonies were present on all the plates on Sabouraud's medium with a pH of 5.5. The bacterial colonies were decidedly fewer in number on the Sabouraud's plates with a pH of 4.0, while on the dextrose-yeast-extract medium with a pH of 4.0, no bacterial growth was demonstrated. The above experiments indicate that the liquid dextrose-yeast-extract medium with a pH of 4.0 is superior to the solid Sabouraud's medium for the isolation of fungi from the sputum.

The dextrose-yeast-extract medium here described is composed of a few simple ingredients, is easily prepared, and affords a ready means for the recovery of fungi from the sputum.

AN EFFICIENT DISSECTING AND OPERATING TABLE*

MAX L. SWEAT, LOGAN, UTAH

THERE is need for an efficient dissecting and operating table. The one built by us has particular advantages over those now on the market. Its advantages are: (1) The mechanical construction throughout is of utmost simplicity. (2) The whole mechanism is of metal. (3) The table top can be instantly adjusted to any plane—slightly short of vertical. (4) The stand may be adjusted for height. (5) The table may be used for either dissecting or operating. (6) The animal to be dissected can be rigidly fastened to the table in only a few seconds, and once in position the natural tension of its limbs prevents any loosening of the hold. (7) Both the stand and the table top can be sterilized.

When dissecting, the hooks are inserted directly into the animal's legs; and when operating, they are inserted into adhesive tape wrapped around the lower part of the legs. Several mechanical devices for holding the legs of animals without harming them were investigated, but the adhesive tape was

*From the Department of Bacteriology and Biochemistry, Utah State Agricultural College. Received for publication, April 22, 1940.

found the most practical. It neither harms the animal nor requires tedious adjusting, and is easily removed after the operation by means of ether.

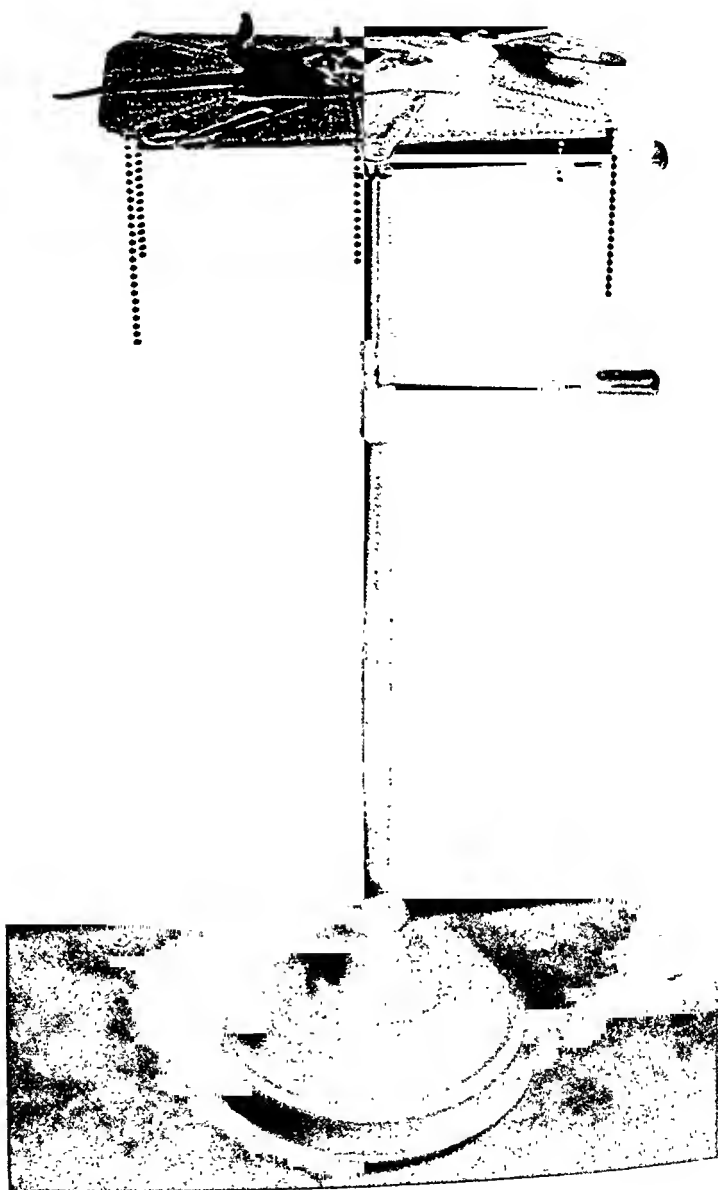


Fig. 1.

DESCRIPTION

1. *Table Top.* This is made of 18 gauge stainless steel (Fig. 1). The top is reinforced with $\frac{1}{2}$ inch 90° angular strips of the same metal running around the bottom 1 inch from the edges. The size 10 by 14 inches is suitable for guinea pigs, rats, and smaller animals. Heavier gauge and larger dimensions are required for tables to accommodate large animals. Adjustments are such that

even a small mouse may be fastened to a large table, but unless work on many size animals is to be done it is advisable to make the table near the size of the animal to be used. Around the edges of the table are V-shaped grooves $\frac{3}{16}$ inch deep and 1 inch apart. These serve to hold the bead chain in tension against the animal. Fig. 1 shows the retractors, hooks, and chains in position on the table top.

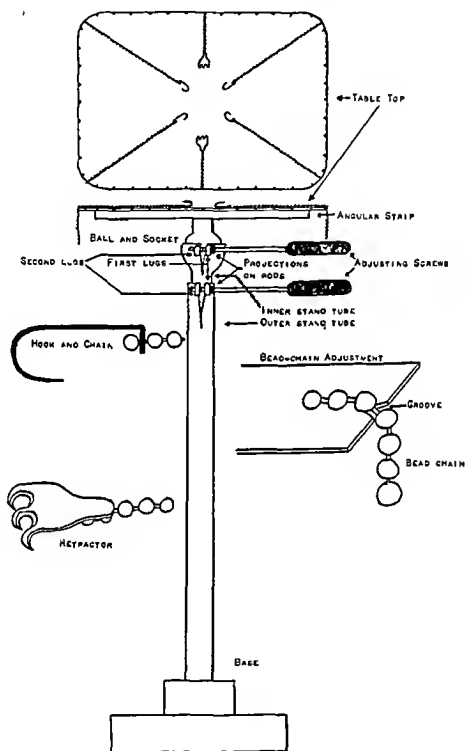


FIG. 2.

2. *Ball and Socket.* The ball is fastened to a rod welded on the bottom of the table top. The split socket is fastened to the inner stand tube. The ball and socket enables the tipping of the table in any direction. This is done by loosening or tightening the adjusting screw. A two inch ball and socket serves well for a table of this size.

3. *Adjusting Screws.* The screws are simply threaded $\frac{3}{8}$ inch metal rods with handles. Just above the threads a projection is welded to keep the rod from going past its threads in the first lug which is slightly larger than the rod

and contains no threads. This lug is soldered on the right of either the slit of the socket or outer stand tube. The second lug, which is soldered on the left of the split, has threads of the same size as the rod. By turning the rod, the distance between the welded projection and the second lug is shortened, thus decreasing the size of the socket or outer tube as the case may be.

4. *Stand Tubes.* These are two; the inner, $1\frac{5}{16}$ inches; the outer, $1\frac{1}{2}$ inches in diameter. Both are 2 feet long. Ordinary chromium-plated brass plumbing tubing serves well. By loosening and tightening the adjusting screw, the inner tube may be raised or lowered to the desired height.

5. *Base.* This is made of two pieces of cast iron. The smaller, 2 by 4 by 4 inches, is fastened by bolts to the larger, 2 by 10 by 10 inches. The stand tube is welded to the base.

6. *Hooks.* These are made from ordinary No. 6 bait fishhooks by filing off the prongs and bending the eyes to right angles, and are then fastened to the chains.

7. *Retractors.* They may be made from 22 gauge stainless steel. They are used in the same manner as ordinary dissecting retractors.

8. *Bead chain.* This is the ordinary bead or ball chain used on light strings, sink stoppers, and key chains.

It will be noticed there is no device on this apparatus for holding the heads of animals. The hooks may be used for this if the animal is to be dissected; if to be operated on, a wide piece of adhesive tape with a hole corresponding to the point of incision in the animal's head may be wrapped entirely around the head and hooks may be inserted in the tape.

I wish to acknowledge the helpful advice and friendly criticism of Dr. J. E. Graves.

SOME IMPROVED METHODS FOR STAINING VAGINAL SMEARS*

GEORGE N. PAPANICOLAOU, M.D., PH.D., NEW YORK, N. Y.

ABOUT three years ago Mrs. Dorothy Fuller, who was at that time working with Dr. Florence Sabin at the Rockefeller Institute, gave me a series of vaginal smears for examination. These smears were stained by Mrs. Fuller with one of Foot's modifications of Masson's trichrome method.¹ This method was briefly as follows:

Iron alum solution in oven 45° to 50° C. five minutes. Wash. Hematoxylin in oven five minutes. Picric acid five to ten minutes. Wash. Ponceau de xyldine-acid fuchsin five minutes. (Fuller used a solution of equal parts of ponceau de xyldine-acid fuchsin and 1 per cent acetic acid.) Rinse in distilled water. Phosphomolybdic acid five minutes. Rinse in distilled water. Light green five minutes. Rinse in distilled water. Acetic acid two minutes. Rinse in tap water, alcohols, and xylol. Mount in balsam.

Smears stained by this method showed a very good differentiation of the cornified cells. A double coloration of these cells was obtained; some cells

*From the Department of Anatomy, Cornell University Medical College, New York. This study has been aided by the Committee for Research on Sex Problems of the National Research Council.

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were pink, some were orange, and others took on intermediate shades. Further experimentation was, however, necessary for evaluating the meaning and the importance of this differential staining of the cornified cells.

Unfortunately, the foregoing method was too long and complicated to be adopted as a standard method for staining vaginal smears. Another disadvantage was its failure to secure a differential staining of the mucous cells, which are characteristic of the early estrous stage in some lower mammals, more particularly in the guinea pig. This disadvantage would not, however, apply to human vaginal smears or to smears of such animals in which the mucous cell type is absent.

The eosin-water blue staining method which I suggested in 1933² permits a good differentiation between the basal cells which are stained blue, the mucous cells which are stained purple, and the cornified cells which are stained with various shades of eosin red. This method consists in treating vaginal smears, previously stained in hematoxylin, with a 0.5 per cent aqueous solution of eosin (Grübler) for four minutes and then, after rinsing in water, in a 0.5 per cent aqueous solution of water blue (Grübler) for about one minute. Shortening of the treatment with water blue would tend to intensify the staining of the cornified elements.

This staining method, though relatively short and simple, could be further simplified through a combination of eosin and water blue. The following mixture gives satisfactory results*: 1.25 Gm. of eosin (Grübler), 0.5 Gm. of water blue (Grübler), 2 Gm. of phosphomolybdic acid, and 350 c.c. of distilled water. An increase in the amount of eosin would result in sharper staining of the cornified cells.†

Since it was found that a satisfactory combination of eosin and water blue could be achieved through the acidification of the mixture, an increase in the color range of the cornified cells was attempted by the addition of orange G, acid fuchsin, and some other stains. By modifying the percentages of the various stains and by adding the proper amount of acid, various shades of orange, pink, and red were obtained. Aniline blue, light green, and other stains were substituted for water blue. The coloration of the noncornified cells could thus be changed.

After long experimentation with over 400 combinations of various imported and domestic stains, a number of satisfactory preparations were developed, some of which are described here. These preparations offer an advantage in that they can be modified to some extent for the purpose of obtaining suitable differentiation of one or another cell type.

While this work was in progress, Shorr^{3, 4} described two staining methods for vaginal smears, based on a more recent modification of the Masson trichrome method by Foot.⁵ The two methods suggested by Shorr are very satisfactory for human smears and are considerably shorter than the method

*This stain is also satisfactory as a general tissue stain.

†A corresponding mixture consisting of domestic stains (National Aniline and Chemical Co.) can be prepared as follows: Aniline blue, water soluble, 60 c.c. of a 0.5 per cent aqueous solution; yellow eosin, 300 c.c. of a 0.5 per cent aqueous solution; phosphomolybdic acid, 1.65 Gm. For intensifying the staining of the cornified cells, the relative amount of eosin should be raised.

used by Fuller. The second of the two methods⁴ takes about six minutes after staining with hematoxylin, but it requires the following steps: (1) one minute in 1 per cent Biebrich scarlet and 0.4 per cent orange G; (2) rinsing in water; (3) one minute in equal parts of phosphotungstic and phosphomolybdic acids; (4) rinsing in water; (5) two minutes in 0.25 per cent fast green FCF; (6) one minute in 1 per cent acetic acid. With the counterstains described herein the staining takes about two minutes and consists of only one step.

A. Solutions made of partly imported and partly domestic stains.

I. Stain DA 15.

Light green F S	Grübler	0.5% Solution	20 c.c.
Orange G	Grübler	0.5% Solution	20 c.c.
Acid fuchsin	Grübler	0.5% Solution	20 c.c.
Eosin yellowish	National Aniline and Chemical Co.	0.5% Solution	40 c.c.
Acid phosphomolybdic	Merck		0.45 Gm.

II. Stain CY 18.

Wasserblau 6B extra	Grübler	0.5% Solution	10 c.c.
Orange G	Grübler	0.5% Solution	25 c.c.
Acid fuchsin	Grübler	0.5% Solution	25 c.c.
Eosin yellowish	National Aniline and Chemical Co.	0.5% Solution	60 c.c.
Acid phosphomolybdic	Merck		0.75 Gm.

III. Stain DF 11.

Aniline blue, water soluble	Grübler	0.5% Solution	12 c.c.
Orange G	Grübler	0.5% Solution	25 c.c.
Acid fuchsin	Grübler	0.5% Solution	25 c.c.
Eosin yellowish	National Aniline and Chemical Co.	0.5% Solution	60 c.c.
Acid phosphomolybdic	Merck		0.6 Gm.

B. Solutions made of domestic stains.

IV. Stain DA 19.

Light green SF yellowish	National Aniline and Chemical Co.	0.5% Solution	12 c.c.
Orange G	National Aniline and Chemical Co.	0.5% Solution	24 c.c.
Acid fuchsin	National Aniline and Chemical Co.	0.5% Solution	20 c.c.
Eosin yellowish	National Aniline and Chemical Co.	0.5% Solution	40 c.c.
Acid phosphomolybdic	Merck		0.45 Gm.

V. Stain DF 20.

Aniline blue, water soluble	National Aniline and Chemical Co.	0.5% Solution	16.5 c.c.
Orange G	National Aniline and Chemical Co.	0.5% Solution	25 c.c.
Acid fuchsin	National Aniline and Chemical Co.	0.5% Solution	25 c.c.
Eosin yellowish	National Aniline and Chemical Co.	0.5% Solution	60 c.c.
Acid phosphomolybdic	Merck		0.7 Gm.

VI. Stain DF 32.

Aniline blue, water soluble	National Aniline and Chemical Co.	0.5% Solution	12 c.c.
Orange G	National Aniline and Chemical Co.	0.5% Solution	25 c.c.
Acid fuchsin	National Aniline and Chemical Co.	0.5% Solution	21 c.c.
Eosin yellowish	National Aniline and Chemical Co.	0.5% Solution	42 c.c.
Phosphotungstic acid	Merck		0.1125 Gm.
Phosphomolybdic acid	Merck		0.225 Gm.

The solutions I to VI are more appropriate for staining human vaginal smears. For lower mammals or rodents, an increase in the percentage of the blue or green basophilic stain secures a better differentiation of the basal cells.

The complete staining procedure is as follows:

1. Smears are fixed immediately (before drying) in equal parts of 95 per cent alcohol and ether* (original method of Stockard and Papanicolaou⁶). Rinse in 70 per cent and 50 per cent alcohols and in distilled water.

2. Ehrlich's hematoxylin, one to two minutes (other hematoxylin preparations may be used).† Rinse in distilled water. Rinse a few times in one per cent hydrochloric acid (may be omitted). Keep in running water about five minutes or in lithium carbonate (3 drops of a concentrated aqueous solution in 100 c.c.) for one minute. Subsequent staining may be affected if slides are left too long in running water.

3. Rinse in distilled water. Stain in one of the counterstains (I to VI) for two minutes. (Staining for five minutes or even longer is advised when smears are thick.) Rinse in water.

4. Rinse in dioxane ten to fifteen times until smears are clear. Rinse in absolute alcohol and xylol. Mount in clarite, Canada balsam, or gum dammar.

The use of dioxane instead of ascending alcohols for dehydration shortens the staining process and improves the differential staining in human smears. It is less desirable for smears of rodents, since it modifies the staining reaction of the mucous cells.

The staining time may be considerably shortened by the omission of the hematoxylin. Slides are stained as follows:

1. Fix smears in alcohol-ether solution for from one to two minutes. Rinse well in 70 per cent and 50 per cent alcohols, and in distilled water.

2. Stain in DF 50‡ or in one of the other counterstains (I to VI) for two to three minutes. (Staining for five minutes or even longer is advised when smears are thick.) Rinse in water.

3. Rinse in dioxane until clear, then in absolute alcohol, xylol, and clarite. Nuclei are stained red, erythrocytes orange, cornified cells red, pink, or orange, and basophile cells green or blue. The nuclei are not as dark and the cell outlines are not as sharp as with hematoxylin, but the cornified cells are more prominent and the basal cells are more transparent.

When a prompt examination of a smear is desired for diagnostic purposes this short method is of particular advantage, for it requires only about three

*Good fixation may be obtained within one to two minutes, though a fixation of from five to fifteen minutes, depending on the thickness of the smear, is more desirable. Slides may be kept in the fixative for several hours or even days without being harmed. Fixation for more than a week or two may, however, change the staining reactions of some of the cells.

†Harris hematoxylin prepared with domestic hematoxylin and ammonium alum gives very good results.

‡VII. Stain DF 50 (particularly adapted to staining without hematoxylin).

Aniline blue, water soluble	National Aniline and Chemical Co.	0.5% Solution	16 c.c.
Orange G	National Aniline and Chemical Co.	0.5% Solution	17 c.c.
Acid fuchsin	National Aniline and Chemical Co.	0.5% Solution	23 c.c.
Eosin yellowish	National Aniline and Chemical Co.	0.5% Solution	4½ c.c.
Phosphomolybdic acid	Merck		0.2 Gm.
Phosphotungstic acid	Merck		0.2 Gm.

to four minutes. It was developed in cooperation with Dr. Ralph C. Benson, of the Department of Gynecology, Cornell Medical College, who finds it especially suitable for preliminary examinations at the Woman's Clinic of the New York Hospital.

The method can be further simplified by the use of dropping bottles instead of staining jars. The stain is poured over the smear, as in bacteriologic staining. The use of dropping bottles prevents evaporation or dilution of the stain and makes its periodic replacement unnecessary. In washing the slides, fluids should be poured on the reverse side so as not to wash off the smear.

The differential staining of the cornified cells is of value in interpreting human vaginal smears in both normal and pathologic cases. Totally cornified cells, like the so-called eosinophilic cells of rodents, show a strong affinity to the orange color. The term "eosinophilic" thus appears to be inappropriate for specifying these cells. The term "acidophilic" or "orangeophilic" would be more accurate. Cells of this type are found in the human vagina more frequently during premenstruum or during pregnancy.²

In the normal cycle the orange shades tend to predominate during the second half of the cycle. Some local irritations or infections, abortions, and certain pathologic conditions associated with bleeding are characterized by a prevalence of pink or red shades. The cornified cells, which usually appear in large numbers after a *Trichomonas* infection, show a preference for orange shades. A similar preference is shown by the cornified cells which follow a prolonged estrogenic treatment. Drying of the cells modifies their staining reactions. For this reason, dry smears cannot be satisfactorily stained with the afore-mentioned methods. Most dry cells take an orange or yellowish color. This may often be a source of error in evaluating smears which were partially dried up prior to fixation.

The general adoption of a standard staining method for vaginal smears was advocated by Schneider.⁷ This would undoubtedly result in a more uniform description of vaginal smear findings by various workers in the field. It would be, however, difficult to reach a general agreement on the superiority of any one of the staining methods which have been thus far proposed. Besides, each one of these methods will probably offer some specific advantage over the others. The DF 50 stain without hematoxylin may be recommended for its shortness, but it does not stain nuclei as well as hematoxylin. This, though a disadvantage, may be of real advantage in the study of certain smear types. It keeps the leucocytes in the background and makes the epithelial cells stand out more distinctly. It may be used profitably in the evaluation of estrogenic or other endocrine treatments, and of cellular changes occurring during the various phases of the sex cycle. For gynecologic diagnostic work* other stains, like DF 32, DF 20, or CY 18 after hematoxylin, would be preferable, since they would bring out more cytologic details.

*The usefulness of the vaginal smear method in gynecologic diagnosis is now being investigated in cooperation with Dr. Herbert F. Traut of the Department of Gynecology.

REFERENCES

1. Foot, N. C.: The Masson Trichrome Staining Methods in Routine Laboratory Use, *Stain Technol.* 8: 101, 1933.
2. Papanicolaou, G. N.: The Sexual Cycle in the Human Female as Revealed by Vaginal Smears, *Am. J. Anat.* 52: 519, 1933.
3. Shorr, E.: A New Technic for Staining Vaginal Smears, *Science* 91: 321, 1940.
4. Shorr, E.: A New Technic for Staining Vaginal Smears. II. *Science* 91: 579, 1940.
5. Foot, N. C.: Useful Methods for the Routine Examination of Brain Tumors, *Am. J. Path.* 14: 245, 1938.
6. Stockard, C. R., and Papanicolaou, G. N.: The Existence of a Typical Oestrous Cycle in the Guinea Pig With a Study of Its Histological and Physiological Changes, *Am. J. Anat.* 22: 225, 1917.
7. Fletcher, P. F.: A Study of the Possible Significance of the Vaginal Smear as an Additional Factor in the Diagnosis of Incomplete Abortion—Discussion by Schneider, P. F., *Am. J. Obst. & Gynec.* 39: 562, 1940.

CHEMICAL

THE SOLUTION AND DIAZOTIZATION OF BILIRUBIN IN CHLOROFORM AND OTHER ORGANIC SOLVENTS*

ROGER S. HUBBARD, PH.D., AND NORMAN HEILBRUN, M.D., BUFFALO, N. Y.

AS PART of a study of bilirubin extracted from serum by chloroform, as described in a recent note,¹ an attempt was made to study the diazo reaction of the bilirubin in such extracts. Difficulty was encountered in obtaining colors comparable with those of the various permanent standards which have been recommended from time to time,²⁻⁴ even when various buffer systems to insure the correct hydrogen-ion concentration were used. Attention was, therefore, turned to the use of standards prepared from bilirubin dissolved in chloroform, as originally recommended by van den Bergh.² The bilirubin used in these experiments† was not easily completely soluble in chloroform. Without the aid of heat, the strongest solutions that could be prepared contained about 10 mg. of bilirubin per 100 c.c. Solutions with concentrations of this magnitude were used by van den Bergh in standardizing the method when it was first described² and have been more recently employed by Malloy and Evelyn⁵ in a photoelectric modification of the technique. By the use of heat, solutions containing 40 to 50 mg. per 100 c.c. could be prepared.‡ The long period of heating sometimes found necessary seemed inadvisable when working with a compound such as bilirubin, and we wished, if possible, to prepare stock solutions containing even higher concentrations of bilirubin than this. Bilirubin in alkaline solvents, both inorganic and organic, were quite unstable. It was finally found that if phenol were added to various organic solvents, very strong solutions of bilirubin could be prepared, and that the stability of such solutions in diffused light was much greater than in the pure solvents. This increased solubility and stability were demonstrated in solutions in chloroform, xylene, acetone, and methyl and ethyl alcohol.

Most of the experimental work was done upon bilirubin dissolved in a mixture of chloroform and phenol, made by dissolving 10 Gm. of phenol in 100 c.c. of chloroform. This solution, prepared freshly before use, would dis-

*From the Buffalo General Hospital and the University of Buffalo Medical School. This work was aided by a grant from the Junior Board of the Buffalo General Hospital. Received for publication, November 1, 1940.

†For the greater part of this work a preparation of bilirubin obtained from the Eastman Kodak Co., was used.

‡In a recent technical bulletin⁶ the use of a stock chloroform solution of bilirubin containing 50 mg. of bilirubin in 100 c.c. is recommended. No directions for preparing this solution are given. The reference furnished is to a paper of Malloy and Evelyn.⁵ In the original article the standard used contained 10 mg. of bilirubin dissolved in 100 c.c. of chloroform. We have not found any other reference to the use of chloroform solutions of bilirubin of this magnitude.

solve the bilirubin used to give concentrations as high as 400 mg. per 100 c.c. without the aid of heat; for the most part the stock solution used contained 250 mg. per 100 c.c.

The problem of diazotizing such solutions was next investigated. In the first experiments the chloroform solution was diluted with methyl alcohol, ethyl alcohol, or acetone until a homogeneous mixture with the water diazo reagent of Thannhauser and Andersen⁷ was obtained. When high concentrations of bilirubin were present, this method was satisfactory, but the amounts contained in chloroform extracts of serum gave colors which were so faint that quantitative measurement of them was difficult or impossible. Various attempts to obtain suitable colors with diazo reagents soluble in ethyl alcohol were unsuccessful, but finally a fairly satisfactory reagent was found. This solution was prepared as follows: 0.1 Gm. of sulfanilic acid was dissolved in 2 c.c. of concentrated hydrochloric acid and 4 c.c. of water. To obtain a satisfactory solution the mixture was heated upon a boiling water bath, and the small lumps of the acid were mashed with a glass rod. The concentrated acid solution was cooled and made up to 100 c.c. with absolute methyl alcohol. The preparation is a saturated solution of sulfanilic acid; some crystals separate on standing. The solution corresponds to solution A of van den Bergh. Solution B was prepared by dissolving 0.5 Gm. of sodium nitrite in 100 c.c. of methyl alcohol. Fifteen minutes before use 0.3 c.c. of solution B was added to 10 c.c. of solution A. Immediately before use one volume of the mixture was diluted with an equal volume of absolute methyl alcohol. The amount of water present in this solution is only slightly less than that which will cause a separation of water when added to chloroform, xylene, or benzene, and this renders the use of absolute alcohol in preparing the reagent necessary.

The diazo reaction was studied upon dilutions of the strong bilirubin solution already described. These dilutions were made with pure chloroform. The technique used for developing the color was as follows: To 2 c.c. of the chloroform solutions 1 c.c. of the diluted mixed diazo reagent was added and the solutions were mixed. After fifteen minutes the colors were read in a microcolorimeter. To ensure the presence of a maximum degree of acidity, and so uniformity of color, 1 c.c. of a strong (80 Gm. to 100 c.c.) solution of trichloroacetic acid in methyl alcohol was usually added just before the reading was made. As far as could be determined, however, this precaution was not necessary in the bilirubin solutions used as standards or in the chloroform blood extracts, for soluble buffer compounds were not present in concentrations great enough to affect the color. If fairly large amounts of a suitable buffer, such as ammonium acetate, were present, the color, which was normally a purple, had a marked reddish tint, and this source of error could be avoided or greatly lessened by the addition of trichloroacetic acid after diazotization.

The method could be successfully applied to solutions containing from 25 to 0.1 mg. of bilirubin per 100 c.c. of chloroform, i.e., in dilutions of the stock solution ranging from 1:10 to 1:300. When higher concentrations were present, the color obtained was too dark to be read in the colorimeter, and weaker solutions gave colors which were too faint to be measured accurately. Within the

range given the relationship between the color obtained and the amount of bilirubin present was linear, i.e., each solution read correctly when matched against those containing twice or one-half as much bilirubin.

Since the stock solution contained phenol, it was necessary to investigate the development of color when phenol solutions in chloroform were diazotized by the technique described. When such solutions contained 10 per cent of phenol, a greenish tint developed slowly. When 5 per cent or less phenol was present, no color could be observed when the method was carried out, nor could any measurable effect upon the weakest solutions of bilirubin studied be distinguished. Since the maximum phenol concentration in any of the solutions used was 1 per cent, there could have been no significant effect of phenol upon the results reported.

Although the actual amounts of phenol in the diazotized mixtures could not have measurably affected the colors obtained, it seemed possible that some reaction might occur between phenol and the bilirubin in the stock solution which would affect the color obtained upon diazotization. To exclude such a source of error, chloroform dilutions of the stock solution were made both soon after it had been freshly prepared and after it had been kept for months in the ice-box. These were diazotized simultaneously with solutions of bilirubin prepared in various ways. These control standards included very dilute solutions of bilirubin in pure cold chloroform, stronger chloroform standards prepared by the aid of heat, and solutions prepared by dissolving bilirubin in water containing minimum amounts of alkali. In the last instance the diazo procedure described above could not be used. These solutions were diluted with alcohol, and the chloroform-phenol solution was diluted with alcohol until the solution was miscible with water and the water-soluble diazo reagent of Thannhauser and Andersen could be used. Precautions were taken to insure the presence of equal concentrations of water, alcohol, and chloroform in the dilute solutions finally studied. No qualitative or quantitative differences in the color given by the reaction could be detected when bilirubin solutions prepared from the stock containing phenol were compared with bilirubin prepared in these three ways.

It seemed desirable to determine whether the colors given by different bilirubin preparations on diazotization were strictly comparable with each other. Many different lots of bilirubin obtained from the Eastman Kodak Co. gave identical results when treated by the technique outlined. A specimen of bilirubin from the Wilson Co., which had a somewhat different consistency from that used in most of the study, and one imported from Germany marked "Bilirubin Homberg," manufactured by Chemische Pharmazeutische A. G. Bad Homburg according to the directions of von Bergman and Eilboth, also gave colors which were the same qualitatively and quantitatively as those obtained from the bilirubin purchased from the Eastman Kodak Co.

The length of time during which the standard would not deteriorate under ordinary working conditions was investigated. The stock solution decomposed rapidly in direct sunlight, but even in clear glass the solution kept its strength for about six months when exposed daily for two or three hours to diffused daylight and artificial light in the laboratory if placed in a cupboard or icebox.

when not in use. A slight darkening of the initial light yellow tint took place, but the intensity of the color obtained upon treatment with diazo reagents remained unaltered. This finding was confirmed repeatedly with stock solutions of various concentrations. The test used was to compare the color obtained from the old bilirubin standard with that given by one weighed and dissolved immediately before use.

Besides these experiments with chloroform solutions of bilirubin, the diazo reaction described was carried out in various other solvents. In aqueous preparations the method was not satisfactory, but in various organic solvents only minor difficulties were encountered. When the stock solution of bilirubin in chloroform and phenol was diluted with benzene, xylene, acetone, ether, or carbon tetrachloride, the results were practically identical with those obtained when chloroform was used. The colors were not qualitatively the same in all the solvents, but in each solvent the colors were alike and were strictly proportional to the amount of bilirubin present. The lowest concentration which gave a visible color varied somewhat in the different experiments. With acetone, for example, the color given by a concentration of 0.04 mg. per 100 c.c. could just be distinguished, while benzene gave a slight color in solutions one-half as concentrated. All the solvents were fairly satisfactory when the concentration of bilirubin was 25 mg. per 100 c.c., although in some instances, notably benzene and carbon tetrachloride, the color given by such concentrations was very intense.

Results with absolute ethyl, methyl and butyl alcohol were less satisfactory. Precipitation took place in the higher concentrations with the first two solvents named, and in the butyl alcohol solution the bilirubin showed a marked tendency to separate on the glass of the container. In the range in which bilirubin was soluble the diazotization was satisfactory. The lowest visible color when dilutions were made with butyl alcohol was 0.08 mg. per 100 c.c. The methyl alcohol diazo reagent possessed no advantage over reagents prepared in water when the bilirubin was dissolved in solvents easily miscible with water.

Certain experiments were carried out on the diazotization of bilirubin extracted by chloroform. Bilirubin was dissolved in water containing a minimum amount of alkali and the pigment was extracted with chloroform after acidifying with hydrochloric and acetic acids. When an aliquot of such extracts was diazotized as described, the recovery ranged from 95 to 100 per cent of the theoretical values. Solutions prepared by diluting the stock solution in chloroform and phenol were used as standards in these experiments.

Freshly prepared solutions of bilirubin in alkali were added to serum. After acidifying and extracting with chloroform, the extract was diazotized as described. The recovery ranged from 90 to 95 per cent of the amount added. When normal human serum was acidified and extracted with chloroform, the amount recovered gave colors in some instances which corresponded approximately with those expected if the serum had contained 0.05 mg. of bilirubin per 100 c.c. Approximate measurements of the yellow color, of such an extract by the method of Soffer³ indicated the presence of a higher concentration of bilirubin than did the diazo color. The results suggested that yellow

pigments which were soluble in chloroform and were not diazotized were present in serum. No indication of the presence of a chloroform-soluble diazotizable compound other than bilirubin was obtained. When serum from patients with a marked degree of jaundice was extracted with chloroform, identical results were obtained, within the limits of accuracy of the methods, by the diazo technique and by measurement of the yellow color.

SUMMARY

Solutions containing as much as 400 mg. of bilirubin per 100 c.c. can be prepared by dissolving the pigment in a solution of chloroform containing 10 per cent phenol. Solutions of bilirubin in organic solvents containing phenol are more stable than in pure organic solvents. Phenol, unless present in high concentrations, has no effect upon the color given by bilirubin treated with diazo reagents. Diazotization of bilirubin dissolved in various organic solvents can be successfully accomplished by the use of a diazotizing solution prepared in methyl alcohol.

REFERENCES

1. Heilbrun, N., and Hubbard, R. S.: The Measurement of the Chloroform-Soluble Fraction of Bilirubin in Persons With Jaundice and Its Significance, *J. LAB. & CLIN. MED.* 26: 576, 1940.
2. Bergh, A. A. Hymans van den, and Snapper, J. D.: Die Farbstoffe des Bluteserums, *Deutsches Arch. f. klin. Med.* 110: 540, 1913.
3. McNee, J. W., and Keefer, C. S.: The Clinical Value of the van den Bergh Reaction for Bilirubin in Blood, With Notes on Improvements in Its Technique, *Brit. M. J.* 2: 52, 1925.
4. Rhamy, B. W., and Adams, P. H.: A New Standard for the van den Bergh Test, *J. LAB. & CLIN. MED.* 13: 87, 1927.
5. Malloy, H. T., and Evelyn, K. A.: The Determination of Bilirubin With the Photoelectric Colorimeter, *J. Biol. Chem.* 119: 481, 1937.
6. Klett Summerson Photoelectric Colorimeter, General directions, Detailed analytical procedures, Klett Manufacturing Co., New York.
7. Thannhauser, S. J., and Andersen, E. D.: Messung der Bilirubinbestimmung im menschlichen serum. Über die *Deutsches Arch. f. klin. Med.* 137: 179, 1921.
8. Soffer, L. J.: Bilirubin Excretion as a Test for Liver Function During Pregnancy, *Bull. Johns Hopkins Hosp.* 52: 365, 1933.

THE FORMOL-GEL REACTION*

DESCRIPTION OF A MODIFIED METHOD FOR QUANTITATIVE ESTIMATION OF SERUM GLOBULIN IN HYPERGLOBULINEMIA AND A CRITICAL ANALYSIS OF THE REACTION

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INTRODUCTION

THE formol-gel reaction, first described by Gaté and Papacostas,¹ is a test for hyperglobulinemia regardless of the cause. It is performed by adding 2 drops of approximately 40 per cent formaldehyde solution to 1.0 c.c. of blood serum. Either solid gelification (Gaté-Papacostas test), or an increase in opacity (Napier's test)^{2,7} occurs in a "positive" reaction after a period of twenty-four hours' standing at room temperature. For a thorough treatment of the development of this test and its modifications, the work of Bing⁸ is recommended.

Wise and Gutman⁹ studied the formol-gel reaction in 113 sera with simultaneous chemical determinations of the several protein fractions. According to their criteria, a test was considered positive when it showed either an *increase in viscosity* from 1+ to 4+, as measured by a change from oily flow to a solid gel, or an *increase in opacity* from 1+ to 4+, as measured by a change from faint haziness to complete opacity. Almost invariably a positive reaction occurred when the globulin level was 4.0 per cent or above, and a negative reaction occurred at globulin levels below 3.4 per cent. Sera containing 3.4 to 4.0 per cent globulin, however, did not give a positive test consistently.

De Vries¹⁰ disregarded the opacity type of reaction and studied 28 sera which showed solid gelification. He concluded that the reaction was usually negative when the globulin level was below 3.6 per cent, and positive when above 3.7 per cent. He also noted that when sera were diluted with isotonic saline below globulin levels of 3.6 per cent gelification no longer took place.

The role of albumin in the formol-gel reaction has been discussed by various workers,⁸⁻¹⁰ the consensus of opinion being that albumin plays a minimal part, if any, in the reaction. The most conclusive studies are those of Napier,³ who showed by precipitation reaction and dialysis that the formol-gel test is specific for globulin, and that albumin has no influence.

In the present study the viscosity of formaldehyde-treated sera was measured and correlated with gelification (maximum viscosity change) and with the concentration of serum globulin. A modification of the formol-gel reaction is described for the quantitative estimation of serum globulin in hyperglobulinemia. In addition, observations were made on the relation of opacity to the content of serum globulin.

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METHODS

Serum was obtained from samples of venous blood allowed to clot, the samples being taken during the fasting state. The albumin-globulin separation was performed by a modification of Howe's method,¹¹ employing 22.5 per cent sodium sulfate at 37.5° C. All nitrogen determinations were made by the micro-Kjeldahl method.¹²

Samples of serum containing an elevated concentration of globulin were diluted progressively with saline and allowed to stand at room temperature for twenty-four hours. These mixtures showed progressive decrease in the apparent viscosity from gelification to unaltered viscosity. At one dilution of the formaldehyde-treated serum the air bubbles introduced by shaking the tube did not rise to the surface but remained stationary. Since this phenomenon appeared consistently and was readily detected, it was arbitrarily chosen as the end point ("stationary bubble" end point). Repeated observations revealed that the concentration of serum globulin which correlated with this end point was 3.1 Gm. per cent. It was also noted that when air bubbles rose very slowly in a mixture, the globulin level was between 2.9 and 3.1 Gm. per cent.

The *modified formol-gel reaction* was performed by introducing serum into test tubes of 8 mm. bore in the following amounts: 1.0 c.c., 0.96 c.c., 0.92 c.c. etc., diminishing the quantity of serum in each subsequent tube by 0.04 c.c. Enough saline was added to each tube to make the contents up to 1.0 c.c. After mixing, 2 drops of approximately 40 per cent formaldehyde solution (U.S.P.) were added. The tubes were tightly stoppered, shaken, and allowed to stand at laboratory temperature for twenty-four hours. The tube demonstrating the above-mentioned "stationary bubble" end point was then selected, and the globulin content, in grams per 100 c.c. of serum, was calculated by the following formula:

$$\text{Grams per cent globulin} = \frac{3.1}{y}$$

where y equals the fraction of 1.0 c.c. of serum present in the tube showing the end point.

Example: If the "stationary bubble" end point occurred in the tube containing 0.80 c.c. of serum, the calculated globulin con-

$$\text{centration} = \frac{3.1}{0.80} = 3.87 \text{ Gm. per cent.}$$

The opacity of formaldehyde-treated sera was compared after twenty-four hours with corresponding untreated samples. The degree of opacity change was read according to the criteria of Wise and Gutnam.⁹

Direct observations of the viscosity (relative to water = 1) of fresh horse sera, treated as in the modified formol-gel reaction, were made by measuring the rate of flow in an Ostwald pipette¹³ at a temperature of 25° C. Similar observations were made also on corresponding dilutions of untreated sera.

RESULTS

1. *Direct Viscosity Studies on Horse Sera.*—Fig. 1 represents the direct measurements of viscosity made on three horse sera.* The apparent globulin

*Horse serum was used because of its high concentration of globulin, and because large amounts of serum are required for the Ostwald pipette.

content of the various mixtures was calculated from the formula using the "stationary bubble" end point. The mixture at this end point was too viscid to flow through the Ostwald pipette. The highest concentration of globulin in which the viscosity could be measured corresponded to a calculated value of 2.85 Gm. per cent. Analysis of the viscosity curve of the formaldehyde-treated sera shows that as the globulin content increased from 0.0 to 2.5 Gm. per cent, the viscosity rose slowly from 1.0 to 6.5; as the globulin content increased from 2.5 to 2.85 Gm. per cent, there was a remarkable rise in viscosity from 6.5 to 21.8. On the other hand, analysis of the viscosity of corresponding dilutions of untreated sera showed that as the globulin increased from 0.0 to 2.85 Gm. per cent, the viscosity rose only from 1.0 to 1.7.

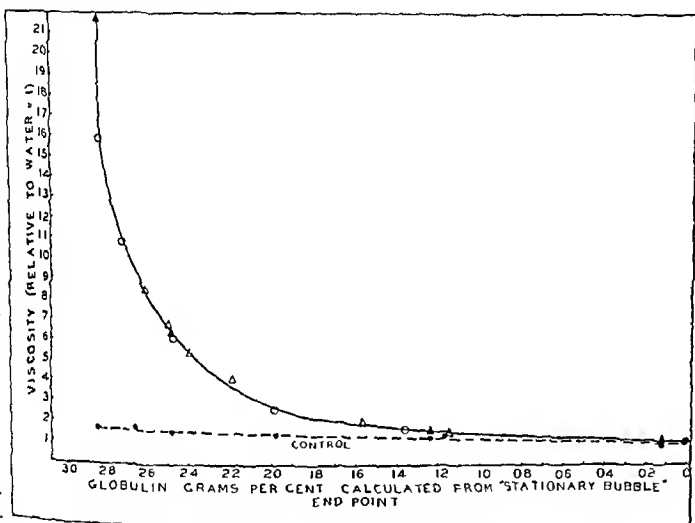


Fig 1.—Direct measurements of viscosity on three horse sera twenty-four hours after the addition of formaldehyde.

The "stationary bubble" end point apparently corresponded to a definite but not measurable viscosity value, since diluted samples in which the viscosity could be measured followed a constant type of curve.

2. *Application of the Modified Formol-Gel Reaction.*—Eighteen human sera and one horse serum (Table I) showing the "stationary bubble" end point (or changes close to the end point) were studied using the modified formol-gel reaction. By application of the formula it was possible to calculate the apparent globulin content of 13 of these sera. In the other 6 sera in which the end point was not obtained, the globulin was estimated to be 2.9 or 3.0 Gm. per cent (see Methods). The globulin values obtained by the modified formol-gel reaction are compared with those obtained by the Howe method. The differences varied from 0.01 to 0.57 Gm. per cent, with an average of 0.22 Gm. per cent for all cases.

TABLE I

CASE NO.	HOWE METHOD-MICRO-KJELDAHL			FORMOL-GEL REACTION	DIFFERENCE IN GLOBULIN (GM. %)	DAYS BETWEEN DETERMINATIONS	CLINICAL DIAGNOSIS
	TOTAL PROTEIN (GM. %)	SERUM ALBUMIN (GM. %)	SERUM GLOBULIN (GM. %)	SERUM GLOBULIN (GM. %)			
1	5.53	2.02	3.51	3.5	-0.01		Alcoholic cirrhosis, ascites
2	8.11	4.70	3.41	3.4	-0.01		Rheumatic heart disease
3	6.98	3.49	3.49	3.7	+0.21	2	Gonococcus arthritis
3a	7.31	3.38	3.93	3.7	+0.23		
4	7.00	3.62	3.38	3.7	+0.32	4	Gonococcus arthritis
4a	7.37	3.25	4.12	3.9	-0.22		
5	5.73	1.85	3.88	3.9	+0.02		Alcoholic cirrhosis, ascites
6	5.83	2.42	3.41	3.1	-0.31	7	Carcinoma of prostate with metastasis, ascites
6a	5.50	2.39	3.11	3.0	-0.11		Hepatitis
7	6.54	2.57	3.97	3.9	-0.07		Catarrhal jaundice
8	7.27	4.07	3.20	2.9	-0.30	5	
8a	6.91	3.96	2.95	3.0	+0.05		
9	6.02	2.55	3.47	2.9	-0.57	5	Generalized anasarca, beriberi heart
9a	5.63	2.49	3.14	3.0	-0.14		Rheumatic heart disease, pericarditis
10	6.23	3.59	2.64	3.0	+0.36		Rheumatic heart disease, pericarditis
11	6.57	3.63	2.94	3.1	+0.16		Normal horse
12	6.22	2.50	3.72	3.5	-0.22		Syphilis, cirrhosis, jaundice
13	7.33	3.98	3.35	3.9	+0.55	4	
13a	7.40	3.92	3.48	3.7	+0.22		

a Represents determinations repeated on the same patient.

TABLE II

CASE NO.	HOWE METHOD—MICRO-KJELDAHL			FORMOL-GEL REACTION	DAYS BETWEEN DETERMINATIONS	CLINICAL DIAGNOSIS
	TOTAL PROTEIN (GM. %)	SERUM ALBUMIN (GM. %)	SERUM GLOBULIN (GM. %)	OPACITY RANGE (1+ to 4+)		
1	6.83	3.99	2.84	++		Carcinoma of breast with metastasis
2	7.53	4.66	2.87	+++		Rheumatic heart disease
3	6.31	4.01	2.29	+		Coronary thrombosis
4	6.52	3.93	2.59	+		Multiple myeloma
5	7.18	4.72	2.46	++		Coronary thrombosis
6	6.28	3.96	2.32	++	19	Diabetes mellitus
6a	6.75	4.45	2.30	+		
7	6.08	3.24	2.84	++		Normal horse used for bleeding
8	3.44	1.44	2.00	++	4	Nephrotic stage of glomerulonephritis*
8a	3.13	1.33	1.80	++		Intercapillary glomerulonephritis*
9	4.28	1.86	2.42	++		
10	6.50	5.05	1.45	+	3	Normal
10a	6.68	4.49	2.19	+		
11	6.50	4.75	1.75	+		Normal
12	6.16	4.52	1.64	+		

*Opacity readings were difficult in Cases 8 and 9, due to high cholesterol values.
a Represents determinations repeated on the same patient.

In this series the modified formol-gel reaction was used for estimation of globulin in hyperglobulinemia, with an average difference of 0.22 Gm. per cent globulin compared to values obtained by the Howe method. This error is similar to that of the Howe method itself.

3. *Relation of Opacity to Hyperglobulinemia.*—Fifteen sera (Table II) were studied which did not show the "stationary bubble" end point, but did show various degrees of opacity after the usual procedure with formaldehyde. By the Howe method the globulin content of these sera ranged from normal values to moderate elevations. Each of these sera showed some degree of opacity (1+ to 3+) which apparently bore no constant relation to its globulin content.

It may, therefore, be concluded that since opacity changes in formaldehyde-treated specimens are present in sera obtained from individuals with normal globulin values, opacity cannot be regarded as a reliable index of hyperglobulinemia.

DISCUSSION

A modified formol-gel reaction is described for estimating the globulin content of sera in hyperglobulinemia. This method has a decided advantage over the standard formol-gel reaction which can only be used as a qualitative indication of hyperglobulinemia.

The "stationary bubble" end point used in this modified technique has been standardized against the Howe method for globulin determination. Howe's method, however, has definite limitations inasmuch as the usual salting-out procedures produce incomplete separation of the albumin and globulin fractions.^{14, 15} The method devised by Tiselius¹⁶ precludes this source of error. Hence, its use in the standardization of the "stationary bubble" end point should result in further accuracy of the modified formol-gel reaction.

The close relationship between the degree of viscosity and the calculated globulin content strongly suggests that viscosity measurements may be used for estimation of globulin.

We gratefully acknowledge the assistance we have received from the following members of the Thorndike Memorial Laboratory: Miss Margaret A. Adams, for the chemical studies on proteins; Miss Constance Brooks, for the viscosity experiments; and Drs. T. H. Ham, W. T. Salter, and F. H. L. Taylor, for their interest and valuable suggestions and criticisms.

REFERENCES

1. Gaté, J., and Papacostas: Une nouvelle réaction des sérums syphilitiques; formol-gelification, *Compt. rend. Soc. de biol.* 83: 1432, 1920.
2. Napier, L. E.: A New Test for Kala-azar, *Indian M. Gaz.* 56: 338, 1921.
3. Ibid.: A New Serum Test for Kala-azar, *Indian J. M. Research* 9: 830, 1921.
4. Ibid.: Practical Experience With Aldehyde Test, *Indian M. Gaz.* 58: 104, 1923.
5. Ibid.: New Serological Test for Kala-azar, *Indian M. Gaz.* 62: 362, 1927.
6. Ibid.: Critical Examination of Antimony Tests for Kala-azar, *Indian M. Gaz.* 63: 687, 1928.
7. Ibid.: Notes on the Significance of Antimony Test in Diagnosis of Kala-azar, *Indian M. Gaz.* 64: 669, 1929.
8. Bing, J.: The Formol-Gel Reaction and Other Globulin Reactions, *Acta med. Scandinav.* 91: 336, 1937.
9. Wise, C. R., and Gutman, A. B.: The Formol-gel Reaction. A Convenient Preliminary Test for Hyperglobulinemia, *Am. J. M. Sc.* 194: 263, 1937.
10. de Vries, A.: On the Connection Between the Formol-gel Reaction and the Blood Proteins, *Acta med. Scandinav.* 99: 425, 1939.
11. Howe, Paul E.: The Use of Sodium Sulphate as the Globulin Precipitant in the Determination of Proteins in Blood, *J. Biol. Chem.* 49: 93, 1921.
12. Peters, J. P., and Van Slyke, D. D.: *Quantitative Clinical Chemistry. Methods.* Baltimore, 1932, Williams & Wilkins Co., p. 691.
13. Findlay, A.: *Physical Chemistry for Students of Medicine*, London, 1927, Longmans, Green & Co, p. 27.

14. Butler, A. M., and Montgomery, H.: The Solubility of the Plasma Proteins. I. Dependence on Salt and Plasma Concentrations in Concentrated Solutions of Potassium Phosphate, *J. Biol. Chem.* 99: 173, 1932.
15. Butler, A. M., Blatt, H., and Southgate, H.: The Solubility of the Plasma Proteins. II. Dependence on pH, Temperature, and Lipid Content in Concentrated Solutions of Potassium Phosphate and Application to Their Separate Precipitation, *J. Biol. Chem.* 109: 755, 1935.
16. Tiselius, A.: Electrophoresis of Serum Globulin. II. Electrophoretic Analysis of Normal and Immune Sera, *Biochem. J.* 31: 1464, 1937.

METHOD FOR THE QUANTITATIVE ESTIMATION OF ACETONE AND ACETOACETIC ACID IN URINE*

PRELIMINARY REPORT

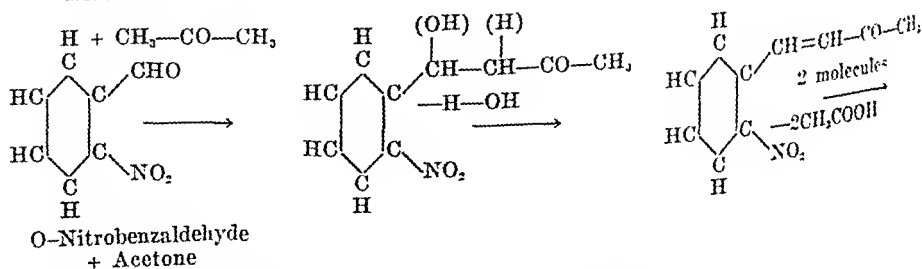
LORRAINE F. NOYES, B.S., EAST PROVIDENCE, R. I.

THOUGH a number of methods for the quantitative determination of ketone bodies have been developed,¹⁻⁷ most of them are too time-consuming and laborious to justify their routine use in the average clinical laboratory. A definite need exists for a simple quantitative method of determining acetone and acetoacetic acid with ketoses due to diet or disease. To meet partially the need for a quantitative method for measuring ketone bodies in patients receiving ketogenic diets, Osterberg and Helmholtz⁸ modified a qualitative method for acetone and acetoacetic acid which allows a roughly quantitative estimation of beta-hydroxybutyric acid. The present report describes a method truly quantitative in its range, requiring no distillation or time-consuming gravimetric procedure, and for which the reagents are readily available. It has been used successfully in the laboratory of the Emma Pendleton Bradley Home for over a year.

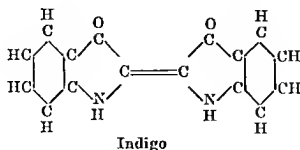
THEORETICAL BASIS

This method is based on the Baeyer and Drewsen⁹ synthesis of indigo. Acetone and ortho-nitrobenzaldehyde, in the presence of sodium or potassium hydroxide, condense to give indigo. As an early means for the synthetic production of indigo this reaction was found to be nearly quantitative. By extracting the indigo produced with chloroform, the condensation may be adapted to the quantitative estimation of acetone and acetoacetic acid as acetone when these are present to a pathologic degree in urine.

Reaction:



*From the Emma Pendleton Bradley Home, East Providence.
Received for publication, August 2, 1940.



While the reaction is quantitative when large amounts of acetone and ortho-nitrobenzaldehyde are present, it is not quantitative when only small amounts of acetone are present. However, the amount of indigo formed is proportionate to the acetone present and to the time of standing. Natural standards must be used, made up at the same time as the unknown, and from acetone solutions of low concentration. Indigo is only slightly soluble in chloroform, so that care must be taken when large amounts of indigo are formed that the dye is completely extracted. Several extractions may be necessary when acetone and acetoacetic acid are present in large quantities.

Too great an excess of ortho-nitrobenzaldehyde or alkali or both will cause secondary reactions, so that the chloroform extract will be greenish and useless for comparison. The solution of indigo in chloroform remains stable for several hours and, if the concentration of indigo is not too great, for several days. Acetaldehyde and pyruvic acid also condense with ortho-nitrobenzaldehyde to give indigo. However, acetaldehyde is never present in urine, and as the method is only useful in the pathologic range, the amount of pyruvic acid present at any time is so small that the error thus introduced is negligible. Other substances present in normal or pathologic urines have not interfered with the reaction either by reacting with the reagents or by preventing the reaction. A review of the literature and practical experience indicate that the condensation is specific for acetone, acetaldehyde, and pyruvic acid.

METHOD

Reagents.—Five per cent solution of ortho-nitrobenzaldehyde* in 95 per cent ethyl alcohol.

Ten per cent potassium hydroxide.

Solutions.—Solutions of acetone containing 1.0 and 0.5 mg. of acetone per cubic centimeter should be made up and accurately titrated with iodine. Standards for urines giving a faintly positive test for acetone and acetoacetic acid should be made from 1 or 2 c.c. of the 0.5 mg. per cubic centimeter of standard, and for those giving a strongly positive test from the 1.0 mg. per cubic centimeter of standard.

Procedure.—Twenty cubic centimeters of a urine giving a positive test for acetone or acetoacetic acid are transferred to a large test tube fitted with a delivery tube. Fresh cork stoppers or rubber stoppers covered with tin foil must be used. The delivery tube need not be cooled during the time the specimen is in the water bath. The tip of the delivery tube is placed well below the surface of 4 c.c. of distilled water in a small test tube. The sample is immersed in the boiling water bath for ten minutes to precipitate proteins and to convert the

*The ortho-nitrobenzaldehyde was secured from the Eastman Chemical Co.

acetoacetic acid to acetone. The boiling of the water bath is so adjusted that very little vapor is transferred to the receiver. The sample is then cooled to room temperature. The receiver and the delivery tube are rinsed with small portions of distilled water and the washings are added to the sample, which is then measured and filtered.

TABLE I

RECOVERY OF ACETONE AND ACETOACETIC ACID FROM URINE BY NEW METHOD

All urines are those of children between the ages of 6 and 12 years. The results are expressed as milligrams of acetone per 100 c.c. of urine

SPECIMEN	PREFORMED ACETONE AND ACETOACETIC ACID BY NEW METHOD (0.2 MG. ACETONE ADDED TO EACH SAMPLE)	PREFORMED ACETONE AND ACETOACETIC ACID BY METHOD OF BEHRE AND BENEDICT	PREFORMED ACETONE AND ACETOACETIC ACID BY NEW METHOD	ACETONE ADDED	ADDED ACETONE RECOVERED BY NEW METHOD	ADDED ACETONE RECOVERED BY METHOD OF BEHRE AND BENEDICT
Normal	1.08	1.021	--	10.00	9.48	9.52
Normal	0.38	.43	--	20.00	19.76	19.68
Normal	0.46	.58	--	40.00	40.21	39.46
Normal	1.43	1.57	--	40.00	39.93	39.71
Ketogenic urine	--	3.59	3.74	--	--	--
Diet 1.5:1	--	--	--	--	--	--
Ketogenic urine	--	13.23	13.67	--	--	--
Diet 2.5:1	--	--	--	--	--	--
Ketogenic urine	--	40.27	41.82	--	--	--
Diet 3:1	--	--	--	--	--	--
Ketogenic urine	--	113.61	118.43	--	--	--
Diet 4:1	--	--	--	--	--	--
Diabetic urine	--	23.11	22.86	10.00	9.92	9.76
Diabetic urine	--	3.29	3.16	10.00	9.67	9.52

A volume of filtrate equivalent to 10 cubic centimeters of urine is transferred to a large test tube. One cubic centimeter of the 5 per cent alcoholic solution of ortho-nitrobenzaldehyde and 2 c.c. of 10 per cent potassium hydroxide are added to the unknown. It is essential that the reagents be added in this order to prevent the precipitation of the ortho-nitrobenzaldehyde. The unknown and a standard, which is set up at the same time and with the same quantity of reagents, are allowed to stand for one hour. The indigo is then extracted with chloroform. Three cubic centimeters of chloroform are sufficient to extract the indigo when the acetone concentration is low. When much indigo is present, it may be necessary to make several extractions. A separatory funnel is used for the extraction and the extraction will be faster if warm water is added to the unknown. Where the tap water is relatively pure it may be used. The extract is transferred to a calibrated tube containing 2 c.c. of water. By the addition of chloroform, the standard and the unknown are adjusted to approximately the same indigo concentration. Cloudiness of the chloroform extract, often present after standing, may be removed by warming the solution under tap water. Indigo is calculated as acetone per cubic centimeter of chloroform in the standard. Standard and unknown are then compared in the colorimeter.

$$\frac{RS}{RU} \times A = \frac{B \times C \times V}{v} = \text{milligrams of acetone plus acetoacetic acid as acetone in the total specimen.}$$

RS is the reading of the standard, usually 20; RU is the reading of the unknown; A is the milligrams of acetone per cubic centimeter of chloroform of the standard; B is the milligrams of acetone per cubic centimeter of chloroform of the unknown; C is the number of cubic centimeters of chloroform of the unknown; V equals the total volume of the specimen; v equals the volume of the sample.

DISCUSSION

In the upper range of total ketone bodies (over 0.2 per cent) the method was found to be truly quantitative. In the lower range the results were low. It seems probable on the basis of further studies now in progress that the range of this method may be increased and that it may be modified to measure total ketone bodies.

SUMMARY

1. A practical clinical method is presented for the quantitative estimation of acetone and acetoacetic acid in urine.
2. The method is based on the condensation of acetone and ortho-nitro-benzaldehyde in the presence of sodium or potassium hydroxide to give indigo.
3. The method is simple and accurate for the estimation of acetone and acetoacetic acid when these substances are present in the urine to a pathologic degree.

REFERENCES

1. Behre, J. A.: A Rapid Quantitative Method for the Determination of Acetone and Diacetic Acid in Urine, *J. LAB. & CLIN. MED.* 13: 1155, 1923.
2. Behre, J. A., and Benedict, S. R.: A Colorimetric Method for the Determination of Acetone Bodies in Blood and Urine, *J. Biol. Chem.* 70: 487, 1926.
3. Deniges, G.: Sur les fonctions organiques pouvant se combiner au sulfate mercurique. *Cas des acétones*, *Compt. rend. Acad. d. sc.* 126: 1868, 1898.
4. Dengies, G.: Combinaison, recherche, et dosage de l'acétone ordinaire avec le sulfate mercurique, *Compt. rend. Acad. d. sc.* 127: 963, 1898.
5. Hubbard, R. S.: Determination of the Acetone Bodies in Urine, *J. Biol. Chem.* 49: 357, 1921.
6. Van Slyke, D. D.: Studies in Acidosis. VII. The Determination of Beta-hydroxybutyric acid, Acetoacetic Acid, and Acetone in Urine, *J. Biol. Chem.* 32: 455, 1917.
7. Van Slyke, D. D., and Fitz, R.: Studies in Acidosis. VIII. The Determination of Beta-hydroxybutyric Acid, Acetoacetic Acid, and Acetone in Blood, *J. Biol. Chem.* 32: 495, 1917; 39: 23, 1919.
8. Osterberg, A. E., and Helmholz, H. F.: Determination Whether Ketonurine Has Bactericidal Action, *J. A. M. A.* 102: 1831, 1934.
9. German patent 19768 (1882).

A RAPID METHOD FOR THE ESTIMATION OF URINE SUGAR*

MICHAEL SOMOGYI, PH.D., ST. LOUIS, MO.

THE most satisfactory clinical approach for studying the carbohydrate utilization (tolerance) of diabetic patients is the one employed by Naunyn and his school at the turn of the century. Essentially it consists in preparing daily balance sheets of carbohydrate consumption and loss of glucose in the urine, the balance representing the amount of carbohydrate utilized in twenty-four hours.

In clinical and laboratory investigations during the past six years we have used this procedure routinely. In cases in which insulin therapy was applied, we did not stop at estimating the total glucose output for twenty-four-hour periods, but found it necessary to run separate quantitative determinations on every batch of urine collected between insulin injections; the information thus obtained proved to be the best criterion for the adequacy of the insulin dosage. One of the advantages of this procedure is that it can be pursued for any length of time without hardship to the patient, whereas gathering of the equivalent information by serial blood sugar determinations is scarcely tolerable for days and nights in a row.

The large number of sugar determinations in our laboratory (150 to 200 on some days) was feasible only with the aid of a rapid and simple method. We describe herein a procedure that we have been using during the past five years. It requires no more time and skill than a properly performed qualitative test.

The basis of our method is the well-known change of color which takes place when sugars are heated in alkaline solution; the shade which develops in the reaction varies from pale yellow to dark amber, depending upon the concentration of sugar. Our primary task was to devise experimental conditions under which the colors developed would be in the closest possible proportionality to the concentration of the sugar. As additional conditions we stipulated a reasonably short reaction period and sufficient sensitivity to permit the estimation of glucose over a wide range of concentrations.

Without enlarging on details of the exploratory experiments, let it be stated in brief that sodium carbonate as the alkali proved to satisfy the requirements. Glucose quantities varying from 1 to 30 mg. in 5 to 6 c.c. of reaction mixture yielded maximum color intensities when the concentration of the carbonate varied within the wide range of 8 to 15 per cent. The maximum color appeared after heating alkaline glucose solutions in a boiling water bath for seven minutes; prolongation of the heating beyond ten minutes caused a gradual fading of the color. (Boiling over an open flame for one and one-half to two minutes produces

*From the Laboratory of the Jewish Hospital, St. Louis.
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the same colors as heating in the water bath for seven to ten minutes.) In the procedure based upon these facts the following reagents and equipment are required:

Reagents.—

1. A 10 per cent solution of anhydrous sodium carbonate.
2. A series of standard glucose solutions, ranging in concentration from 0.5 to 6 per cent.

The standard glucose solutions are best prepared from a stock solution, a 10 per cent solution of glucose in water, saturated with benzoic acid (0.25 per cent) as a preservative. From the stock a series of 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, and 6.0 per cent standard solutions is prepared by dilution with water containing 0.25 per cent benzoic acid. Stock solution and standards keep indefinitely at room temperature.

Equipment.—

1. Test tubes of 14 mm. inside diameter. All test tubes employed must be of identical diameter. A simple way to select uniform test tubes is to measure accurately 10 or 15 c.c. portions of water into a series of tubes and to retain for use only those in which the water level is of the same height.
2. Pipettes for measuring 0.5 c.c. and 5.0 c.c. portions of fluid.

Procedure.—

Introduce into seven test tubes 0.5 c.c. portions of the standard glucose solutions, and into the other test tubes 0.5 c.c. portions of the urines to be analyzed, then add to every test tube 5 c.c. portions of the sodium carbonate reagent. Mix the contents, place the test tubes in a rack, and heat, immersed in boiling water, for eight minutes. Racks accommodating from one to two dozen (or more) test tubes are serviceable.

After eight minutes' heating read the results without undue delay. To this end place the standards in an ordinary test tube rack, leaving one space between each two tubes free for the matching of the test tubes containing the urine samples. When matching colors, view the test tubes transversely, holding the rack against a window or an artificial source of light. If the color of the unknown does not closely match any one of the standards, but lies between two of them, estimate the intermediate value by interpolation. If, for example, the color shade of an unknown is halfway between the 3.0 and 4.0 per cent standards, its sugar content is 3.5 per cent; if it is between 3.0 and 4.0 per cent, but distinctly nearer the 3.0 per cent standard, then it contains from 3.2 to 3.3 per cent of glucose, etc.

In laboratories where urine sugars are determined throughout the day, the slow but measurable fading of the colors at room temperature constitutes a drawback. One can get along with standards prepared once a day, but with the realization of the fact that analyses performed when the standards are several hours old will give readings that are 10 to 15 per cent too high. This is especially true on hot summer days, when the fading of colors is accelerated by high room temperatures.

Such degree of accuracy seems quite acceptable for a semiquantitative method; but we succeeded in eliminating this source of error by devising permanent standards, in sealed test tubes, which remain unchanged indefinitely. After an extensive search for adequate permanent standards, alcoholic iodine solutions were finally found best suited for the purpose; Dr. Simon Russi and other workers in our laboratory gave valuable assistance in this endeavor.

The stock solution for permanent standards is a 0.01 normal iodine solution in absolute alcohol from which a series of solutions is prepared by dilution with absolute alcohol. The normality of the several diluted solutions is given in Table I. A 0.008 normal alcoholic iodine solution is of the same color, shade, and intensity as the 6 per cent glucose standard and thus takes the place of it; a 0.0058 normal iodine solution is a substitute for a 5 per cent glucose standard, and so on, as given in Table I.

TABLE I
CONCENTRATIONS OF ALCOHOLIC IODINE SOLUTIONS USED AS PERMANENT STANDARDS

NORMALITY OF IODINE SOLUTIONS	CORRESPONDING PERCENTUAL GLUCOSE CONCENTRATIONS
0.0004	0.5
0.0009	1.0
0.0018	2.0
0.0028	3.0
0.0043	4.0
0.0058	5.0
0.0080	6.0

TABLE II
COMPARISON OF RESULTS OF URINARY SUGAR DETERMINATIONS BY THE NEW METHOD, WITH TRUE SUGAR VALUES ESTIMATED BY A COPPER-iodOMETRIC METHOD

NO.	GM. OF SUGAR PER 100 C.C. OF URINE	NEW METHOD
	COPPER-iodOMETRIC METHOD, WITH STAFFER-SOMOGYI REAGENT NO. 50	
1	0.21	0
2	0.42	0.3
3	0.31	0.5
4	0.41	0.3
5	1.2	1.2
6	1.3	1.3
7	2.1	2.0
8	2.6	2.5
9	3.0	3.0
10	3.9	4.0
11	5.0	5.0
12	5.0	5.0

Approximately 5 c.c. portions of these iodine solutions are introduced into pyrex test tubes of 14 mm. inside bore and the tubes are then sealed. In this condition the standards remain unchanged without requiring any special precautions, as for example, protection from light or changes in room temperatures. The permanence can be readily ascertained by comparison with standards freshly prepared with pure glucose solutions. The earliest permanent standards in our laboratory are now over two years old and show no deterioration.

When using the permanent standards with a rather simple comparator block,* the accuracy of the method is great enough to advance it into the rank

*Sets of permanent standards with a matching block were placed on the market by A. S. Aloe Co., St. Louis, Mo., and are available through laboratory supply houses.

of adequate quantitative methods. The accuracy of this procedure is illustrated in Table II, in which results obtained by it are compared with determinations run by the copper-iodometric reagent of Shaffer and Somogyi.¹ It is to be remarked that the results of the copper-iodometric analysis represent true (fermentable) sugar values, that is to say, values that do not include any reducing matter other than sugar. These values, as may be seen, compare favorably with those obtained by the new procedure.

The method, we reiterate, is as rapid and simple as a properly executed qualitative test for urinary sugar, particularly when one is to carry out considerable numbers of determinations. In instances where only occasional single determinations are made, however, preliminary heating of a water bath to boiling and heating of the reaction mixture for eight minutes may involve a waste of time. This difficulty can be overcome by the use of an open flame. The analysis then is carried out as follows:

Measure into a test tube 0.5 c.c. of urine and 5 c.c. of the carbonate reagent, drop in two small glass beads or quartz pebbles, and a drop of paraffin oil (or a corresponding bit of paraffin wax), heat to boiling over an open flame and, turning the flame low, keep the liquid *very gently* boiling for one and one-half to two minutes. The color develops to the same shade as after heating for eight minutes in a boiling water bath and can be matched against the permanent standards.

Since this method consumes no more time and work and requires no more skill than the generally employed qualitative methods, as for example Benedict's, in our institution the quantitative estimation of urine sugar has completely displaced the qualitative tests based upon copper reducing power.

A distinct advantage of the carbonate reaction is that it is more specific for sugars than are copper reagents. This quality comes mainly into play when dealing with concentrated urines, so frequent in hot summer weather, which may reduce appreciable amounts of copper without containing any abnormal amounts of glucose; with our method such urines give negative results.

If the urine is abnormally dark colored or contains blood or bile, it must be decolorized preliminary to the analysis. To this end about 2 to 3 Gm. (roughly one-half teaspoonful) of Lloyd's reagent is added to about 10 c.c. of urine in a test tube or a small flask, the mixture is gently agitated for about a minute (or longer) and then filtered through a dry filter paper. The nearly colorless filtrate is ready for analysis.

REFERENCE

1. Shaffer, P. A., and Somogyi, M.: J. Biol. Chem. 100: 695, 1933.

MEDICAL ILLUSTRATION

LANTERN SLIDES: A NEW TECHNIQUE*

M. MUSCHAT, M.D., F.A.C.S., PHILADELPHIA, PA.

THE use of lantern slides in medical presentations is becoming more and more popular. The success of a presentation, whether long or short, depends largely upon the number and excellence of the slides shown. To the essayist it means a very definite aid in expounding and elucidating the details and fine points of the presentation. The audience is instantly aware and attentive, and remembers well the important part of the subject under discussion.

Our present-day photographic method of preparation of slides is rather uniform. Charts, graphs, tables, and drawings are photographed and positive slides are made. This method is good, but its cost is high and forces the average essayist to limit the number of slides he uses. By thus limiting himself to only a few slides he foregoes the opportunity of stressing many convincing points, leaving the impression of haste and incompleteness.

An additional method is with the use of cellophane, which is available as "announcement sheets" and "radio mats." The cellophane, encased in an envelope, is covered with a sheet of carbon paper. After the material is copied on the typewriter, the envelope is opened, the carbon copy is removed, and the finished film is placed between two cover glasses and taped. This method is simple and inexpensive, but it can only be used for writing a few sentences on a small area. Previous attempts to make charts or drawings on cellophane have ended in failure because of the small area (7.6 by 5.7 cm.) available, the smeary copy, and the inability to erase errors.

I am presenting here a novel method of slide preparation that is easy to handle and is low in cost. It consists of a very thin frosted paperlike film, upon which one can typewrite, draw with a pencil or pen and India ink, and even use water colors with good results. This material can be handled without difficulty, for it keeps its shape and does not become smeary. It possesses all the qualities and characteristics of a fine drawing or tracing paper. After the drawing or typing is completed, the frosted sheet is placed between two glass slides, bound with tape, and is ready for use. Thus an original drawing or chart can be prepared without having to resort to photography.

Material Used.—The frosted film is available on the market as "Traceolene" (light weight only), "Frosted Protectoids" (three weights or thicknesses:

*From the Departments of Urologic Surgery, Mount Sinai Hospital and the Medical School of the University of Pennsylvania, Philadelphia.

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light, medium and heavy), and "Plastacele" (varied thicknesses). In testing various thicknesses their optimum usefulness for specific purposes was found. The results of this study are summarized in Table I.

Typewriting.—The frosted film, cut into strips of 9.2 cm width, is placed in the typewriter. The typing is done on the frosted side. It is better to use a few sheets of ordinary paper behind it, to obtain a sharper print. An extra black ribbon will produce darker print and show up better on the screen. The best print is obtained by placing the film in a folded carbon paper, thus obtaining a double print: front and back. One can utilize the entire surface area of the frosted film, leaving a margin of about 2 to 3 mm. for the binding tape (Fig. 1). The surface area thus available is 55 per cent greater than in the commercial cellophane method marketed as "radio mats." This ratio is shown in Fig. 2.

TABLE I

	LEAD AND CARBON PENCILS	INK	INDIA INK	STAMP	TYPE	PENCIL ERASURES	INDIA INK REMOVAL BY SCRATCH- ING	CLARITY OF TYPE	HAN- DLING
Cellophano Tracelene or frosted: 88 AB	None Poor	Poor Poor	Poor Good	Poor Fair	Fair Good	None Good	Poor Fair	Smeary Clear	Smeary Clean
Frosted: 150 AB	Good	Poor	Good	Fair	Good	Good	Good	Clear	Clean
Frosted: 300 AB	Poor	Poor	Good	Poor	Poor	Good	Good	Clear	Clean

Some projectors and lantern slide carriers are so constructed that they cut in on the sides of lantern slides in which the data extend too far to the right or left. It is advisable, therefore, to have the contents of the slide cover a space of only 7 cm. vertically and 7.5 cm. horizontally. This allows a space on the left of the image for attachment of a label or tab on which are noted the necessary data as to the owner, the nature of the contents, and the number of the slide. The large circle in the lower left-hand corner is an indicator for the projectionist to put the slide in the machine in the proper manner.

When a comparison is made of the print on clear cellophane with that on frosted film, the sharpness of the letter print in the frosted material and the smeary, unclean result on the cellophane can readily be seen. An enlargement of both results illustrates this difference in Fig. 3.

Plain rubber stamping can also be used, as seen in Fig. 4.

If partial legends are required, they should be printed with the proper spacing. The frosted paper is then cut to the correct sizes and placed on the drawing or directly on the slide as needed.

Drawing With Pencil.—In drawing an object on frosted film the same method is employed as when using ordinary paper. I recommend the use of carbon pencils since the marks left by these pencils will show up better on the screen. By using shaders dipped in carbon pencil dust on a sandpaper board, I was able to draw the most delicate shadings desired. Shaders are made by

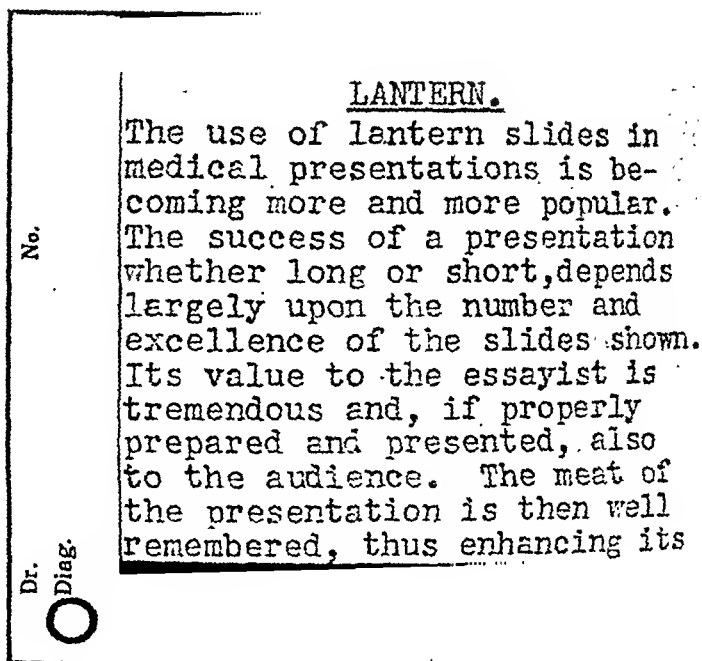


Fig. 1.

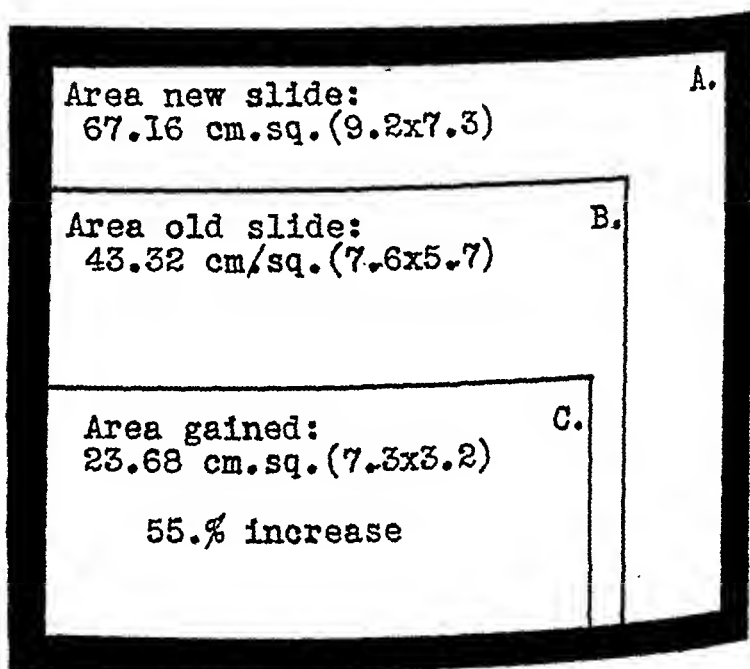


Fig. 2.

Dec. 2 m-stripe
 ed. Ve w, car
 ing-c al a
 s, mar
 ed le d
 uria

Fig. 3

ABCDEFGH
 1234

Fig. 4.

No. _____

Dr. _____

Diag. ☐



Fig. 5.

cutting and sandpapering to a point ordinary erasers and pieces of cork. A sharp, pointed pencil may be used on this frosted material without tearing or wrinkling. The film will stand erasing with hard and soft rubber (Fig. 5).

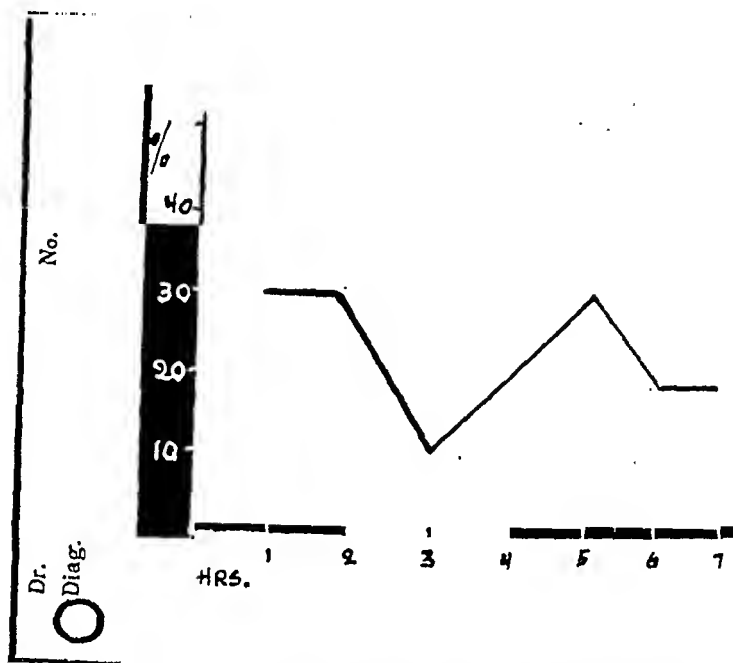


Fig. 6.

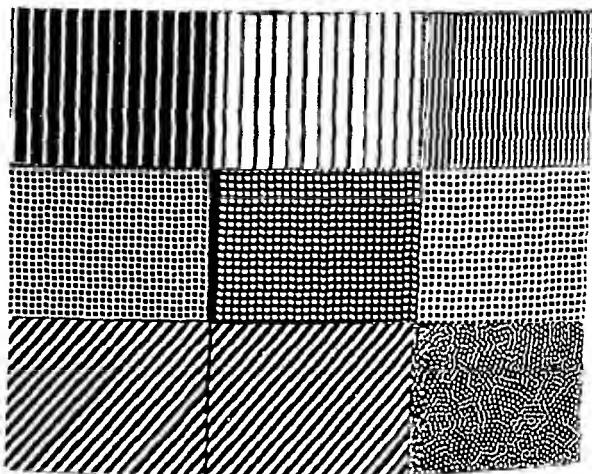


Fig. 7.

Drawing With India Ink.—The finest lines can be drawn on frosted film with pen and India ink. I drew charts and graphs on it the same as I do on fine drawing paper. Brilliant results may be achieved. Scratching out of the ink is easy. Ink smears on the glazed back may be wiped off with a wet sponge (Fig. 6).

Shading of Areas.—For this work I have utilized a special cellophane film with various designs or patterns of dots and lines printed on it. It is called "Transograph Shading Film"—A. Standard nonadhesive, and B. Contak adhesive back (see Fig. 7). Spaces and columns were shaded by pasting them on the frosted film.

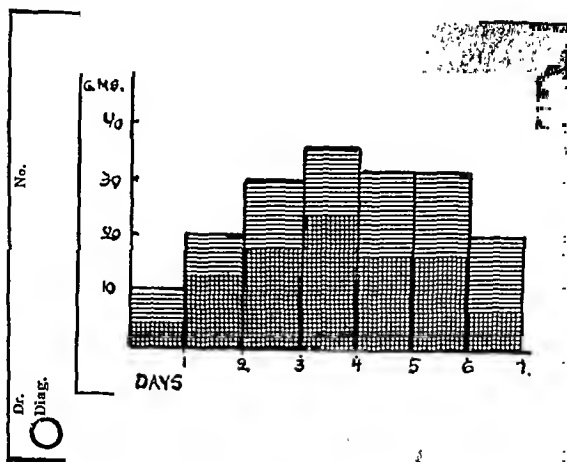


Fig. 8.

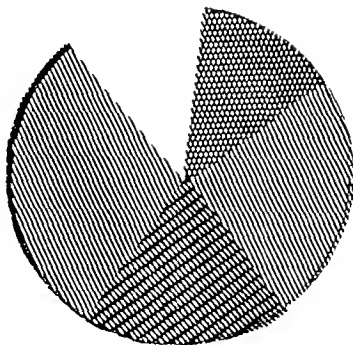


Fig. 9.

The shading film is useful in many ways. When using the Contak with the adhesive back, one side of the film is covered with a white paper upon which one

can draw with pencil or ink. After the required area is marked off, it is cut out. The protective white paper is then lifted off and the pattern is pasted directly on the frosted film. In order to produce varied shadings, the same film is pasted on the first layer of film, with the design running in a different direction. Many different types of shadings can thus be obtained. The handling is very simple and clean, and the final effect on the screen is brilliant (Figs. 8 and 9).

Tracings.—The frosted film is adaptable as a tracing paper in making a tracing of any desired image. By placing it upon the picture and fixing it with Scotch cellulose tape, one can trace any minute detail, either with carbon pencil or India ink. Such tracings can be very useful to those who cannot do freehand drawing.

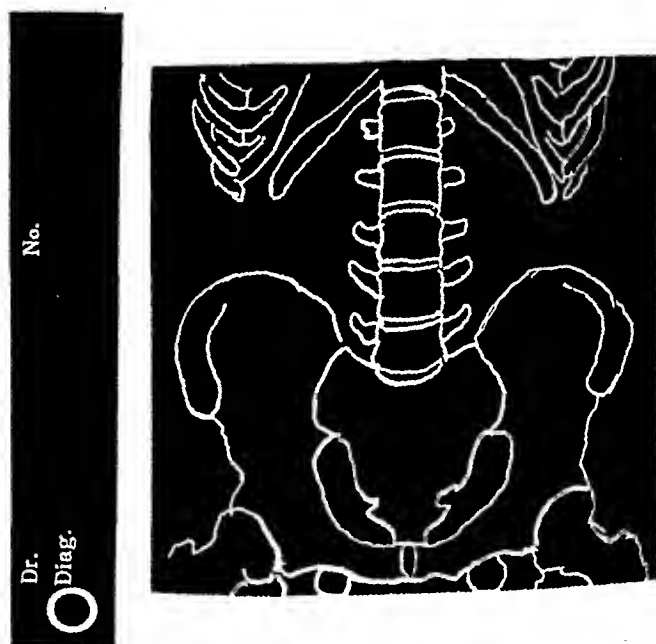


Fig. 10.

Color Slides.—Many desired effects in flat color can be obtained by the use of colored cellophane. The cut-to-shape colored cellophane, pasted on the frosted film, produces an effective color on the screen for differentiation. It can be pasted on the adhesive side of adherent cellophane, thus fixing it in permanent position. Colored cellophane is used for square and round graphs.

Permanency.—Slides made of frosted film are superior to thin cellophane in maintaining their shape and clarity over a long period of time. Under ordinary conditions they will not warp or wrinkle, and the type will not fade.

DEPARTMENT OF ABSTRACTS AND REVIEWS

ROBERT A. KILBUFFE, M.D., ABSTRACT EDITOR

SERUM LIPASE, Diagnostic Significance of Determinations of, Johnson, T. A., and Bockus, H. L. Arch. Int. Med. 66: 62, 1940.

This report comprises data on 616 independent determinations of serum lipase for 371 patients, performed over a period of three years. In the authors' experience, values for serum lipase are significant when they exceed 1 e.c. of a twentieth-normal solution of sodium hydroxide.

A large number of patients with functional and organic disorders, but with no clinical, surgical, or pathologic evidence of pancreatic disease were examined in order to determine the incidence of positive tests in nonpancreatic disorders. Uniformly normal lipase values were obtained in uncomplicated diabetes mellitus, hyperthyroidism, syphilis, tuberculosis, hypothyroidism, venereal lymphogranuloma, nonpancreatic malignant disease, uncomplicated peptic ulcer, gastritis, gastroduodenitis, functional colonopathies, nonobstructive organic diseases of the small and large intestines, and heart disease. The significance of normal values for lipase in these conditions is emphasized in order to stress the specificity of the test as an indication of pancreatic disease.

The study confirms the reports of others that diseases of the pancreas show the highest incidence of increased values for serum lipase. In 9 of 11 patients with acute pancreatitis and in 5 of 8 patients with proved cancer of the pancreas, pathologic values for lipase were obtained. Further proof that disease of the pancreas is the usual cause of elevations in serum lipase is supplied by the data for a group of 60 persons with cholelithiasis. A positive test was obtained in only 1 of 31 patients without jaundice and without a previous history of jaundice, whereas in 31 per cent of the remaining 29 patients with cholelithiasis with jaundice or with a history of jaundice values above 1.0 e.c. were obtained.

The study does not supply any support for the view that hepatocellular injury causes an elevation of the serum lipase. Normal values were obtained in 15 persons with so-called catarrhal jaundice, 2 with toxic or infectious hepatitis, and 12 with hyperthyroidism. These findings tend to throw some doubt on the theory that hepatic dysfunction may disturb the level of the serum lipase. If the findings in cases of catarrhal jaundice are confirmed by others, the lipase test may prove to be of distinct value in certain cases of jaundice of obscure causation, for an elevated value for serum lipase would tend to exclude the diagnosis of catarrhal jaundice.

As might have been anticipated, the authors noted no relation between hyperbilirubinemia and the values for serum lipase. This statement is based on simultaneous determinations of serum lipase and bilirubin on the same blood samples from 44 jaundiced patients. The fact may be considered additional evidence against the theory of a hepatic causation for hyperlipasemia.

In chronic hepatic disease associated with widespread destruction of the hepatic parenchyma, there was a rather high incidence of hyperlipasemia. High values were obtained in 7 of 24 patients with cirrhosis of the liver and in 2 with hepatic tumor. An examination of the material (Table 4) does not supply very convincing evidence that the possibility of pancreatic involvement was satisfactorily eliminated. The pancreas was examined at autopsy in only one patient (9) and was reported to be normal.

Four patients with intestinal obstruction had elevated values for serum lipase. The authors have found no reports of a similar observation in the literature. If the observation is confirmed by others, the serum lipase test will have another clinical application of importance.

The authors feel that this study justifies the conclusion that the serum lipase determination is of considerable clinical importance and that a wider application is warranted than it now enjoys.

SEPTICEMIA, Staphylococcic, Sutherland, R. T. Arch. Int. Med. 66: 1, 1940.

The treatment of staphylococcic septicemia should not be considered hopeless, since by early active treatment the mortality can be reduced.

Patients having active or recently healed carbuncles, furuncles, or blisters, with either prolonged, mounting, or septic temperatures for which no cause can be found; patients with symptoms and signs of a beginning osteomyelitis; and patients with staphylococci in the blood, should immediately have heroic treatment.

At present there is no standard as to what constitutes such treatment.

Experience suggests that in addition to supportive and surgical care, early treatment with large doses of antitoxin in a total dosage, varying from 300,000 to 1,000,000 or more units, together with some bactericidal element such as immune blood, gives encouraging results.

SCHICK REACTION, Effect of Human Serum on the Blood Antitoxin Titer and, Barenberg, L. H., Greenstein, N. M., and Leighton, B. Am. J. Dis. Child. 60: 36, 1940.

Because of the fact that susceptibility of a person to diphtheria can be determined within twenty-four hours by means of the Schick test and blood titration, diphtheria was singled out from other contagious diseases for special study to determine the mechanism by which the routine injection of human serum prevents the development of secondary contagious diseases in a hospital ward.

This investigation is based on observations of the effect of human serum on the cutaneous reaction in 141 children with positive reactions to the Schick test and on the blood titer of 55 of this group. The children ranged in age from 6 months to 12 years. The Schick test was performed on every patient admitted to the hospital, and a retest was given within several days after the serum injection. Subsequent retests, at monthly intervals, were carried out on patients in whom a reversal of the reaction followed serum injection. Intramuscular injection of from 10 to 30 c.c. of human serum was given to every patient within twenty-four hours after admission. Within twenty-four hours after the serum injection a central pallor developed in the area of the Schick reaction, and within forty-eight hours there was a clear demarcation between the central pallor and the peripheral inflammatory zone. In patients to whom serum was not given, this phenomenon did not occur. This anomaly did not indicate that a subsequent reversal of the Schick reaction would take place.

Injection of human serum brought about a reversal of the Schick reaction in 98 children, or 69.6 per cent. In 43 children, or 29.4 per cent, the Schick reaction remained persistently positive despite the serum injection. The intensity of the Schick reaction, however, apparently was reduced by means of the human serum. The modification of the reaction of 8 children who received from 10 to 15 c.c. was similar to that of 24 children who received from 20 to 45 c.c. of serum.

Titration studies were done on 55 children. Of 33 children in whose Schick reaction the serum brought about a reversal there was a significant rise in titer in 91 per cent, while of 22 whose Schick reaction was persistently positive there was only a slight rise in titer in 40 per cent. There was a close correlation between the rise in serum titer and the reversal of the positive Schick reaction. Analysis is presented of the observations on 39 children of the youngest age group, in whom 22 whose Schick reaction became negative after injection of serum had a rise in titer of 166.6 per cent, as compared with a rise of 44.4 per cent in 17 children whose Schick reaction was persistently positive. The average titer of the donors' serum in the former group was 0.0047 unit per cubic centimeter higher than the amount of the serum given to the latter group. These observations would indicate that the higher

potency of the serum was responsible for the reversal of the Schick reaction as well as for the rise in titer of the patients' blood. In this study the repetition of the Schick test was not responsible for the rise in the blood titer or for the reversal of the reaction, as the interval between the second test and the subsequent titration ranged between seven and thirty-three hours, a period in which the Schick material could not be a factor.

Human serum protected more than half of the children for twenty and five-tenths weeks and the remainder for three and two-tenths weeks. The average period of protection was thirteen weeks in the cases of the youngest group of children, as compared with twenty-two and twenty-six weeks in those of the two older groups. It was found that the youngest age group, in which the smallest percentage was protected, lost its immunity sooner.

MONONUCLEOSIS, Infectious, The Histological Lesion in Lymph Nodes in, Gall, E. A., and Stout, H. A. Am. J. Path. 16: 95, 1940.

A survey of the literature reveals the fact that no consistent lesion has hitherto been described in the lymph nodes from patients with infectious mononucleosis. The authors have studied lymph nodes removed from ten such patients at various stages in the illness and have described a characteristic morphologic pattern. This appears with such regularity in this disease and so rarely in other conditions that it is believed to have diagnostic importance.

The basic lesion of infectious mononucleosis is apparently the result of the varied responses of several different elements composing the lymph node to a single, presumably irritative stimulus. The structures manifesting this reaction may be enumerated as follows: (a) lymphoid follicles, (b) lymphoid cords, (c) lymph sinuses, (d) sustentative elements and blood vessels.

The important crude diagnostic feature which serves in the differentiation from primary neoplastic disease of the lymph node is the retention of gross architectural relationships. This is particularly the case with reference to the persistence of subcapsular and radial sinuses.

The basic process underlying the lymph node lesion in this disease is essentially the result of proliferative stimulation of the components of the node.

In what appears to be the early stages of the lesion, the germinal centers of the lymph follicles become hyperplastic and show large secondary nodules. These consist of masses of apparently fused cells with abundant, poorly defined, basophilic cytoplasm and large vesicular nuclei (stem cells), and also of varied numbers of mononuclear elements with more sharply defined and demarcated eosinophilic cytoplasm and eccentrically placed lobulated or reniform nuclei (probably clasmatoocytes). Mitotic figures are numerous and there is evidence of phagocytic propensity among many of the clasmatoocytes.

At the same time there are increased numbers of mitotic figures in the larger cells of the extrafollicular lymphoid substance in both cortical and medullary regions.

During the florid stage of the disease the appearance of the lymph node is quite unusual and presumably specific. The node is enlarged and shows greatly increased cellularity in the medullary, cortical, and sinus substance. The cords are swollen by a rich mixture of small and large lymphocytes, stem cells, and lymphoblasts, infectious mononucleosis cells, and large eosinophilic phagocytic elements. Follicles persist in some cells, but in others they are apparent only as occasional, partially disrupted germinal center fragments, and reticulum stains exhibit a vestige of concentric perifollicular arrangement. There is marked sinus compression and distortion, although identity is preserved. The sinus lumina contain variable numbers of cells similar to those noted in the pulp. Cells of this type are also evident in small numbers in both the trabeculae and capsule of the node. There is an apparent increase in reticulum meshwork fibrils, vascular channels are much more abundant than usual, and vascular endothelium is hyperplastic.

BILIRUBIN, Comparison of Tests for, in Urine, Foord, A. G., and Baisinger, C. F. Am. J. Clin. Path. 10: 238, 1940.

In doing tests for bilirubin in the urine, particularly to avoid missing small quantities, some method of concentration of the pigment should be used, either precipitation and adsorption by barium chloride or adsorption by tale. The most commonly used tests on unconcentrated specimens are not sensitive and often are difficult to read.

The diazo spot method of Godfried is a highly satisfactory method, as are also the Harrison spot and the Naumann methods. The latter is perhaps more time-consuming. The red color developed in the diazo spot method is more easily read than the colors produced by the other methods.

Diazo Spot (Godfried).—Precipitate bilirubin with barium chloride, filter, spread filter paper on dry filter paper, and add 1 drop of diazo reagent, 4 drops of 95 per cent alcohol, and 1 drop of phosphate buffer. Red to pink color denotes a positive reaction. Test works best with small amounts of bilirubin. If precipitate is deep yellow in color, add more of reagents, or better, dilute urine and repeat. A quantitative technique by diazo method is given by this author.

Harrison Spot (Godfried).—Add to 10 c.c. urine 5 c.c. of 10 per cent barium chloride solution, mix and filter. Spread filter paper on dry filter paper. Add 1 to 2 drops of Fouchet's reagent (trichloroacetic acid 25 Gm., water 100 c.c., 10 per cent ferric chloride 10 c.c.). Blue to green color indicates a positive reaction.

Naumann.—Filter through paper in a Buchner funnel of 3.5 cm. diameter 5 c.c. of a 10 per cent emulsion of tale in water. Suck nearly dry. Add 5 c.c. of urine and suck nearly dry. Add 1 drop of Fouchet's reagent or 10 per cent nitric acid in middle of disk. Blue color is positive.

BONE MARROW, Sternal, in Hyperthyroid and Myxedematous States, Jones, R. M. Am. J. M. Sc. 200: 211, 1940.

Sternal marrow aspirated from 18 "normal" individuals contained an average of 6.2 per cent nucleated cells.

Sternal marrow from 12 individuals with hyperthyroidism contained an average of 13.5 per cent nucleated cells, about $2\frac{1}{2}$ (217 per cent) times the "normal" finding.

Sternal marrow from 7 individuals with hypothyroidism contained an average of 2.4 per cent nucleated cells, a little more than one-third (38 per cent) of the "normal" average.

Five patients with hypothyroidism treated with desiccated thyroid or thyroxin, and subsequently studied, showed a marked rise in the percentage of nucleated cells in the sternal marrow.

A patient with thyrotoxicosis following thyroid ingestion showed a marked decrease in the percentage of nucleated cells in the marrow when the thyroid was stopped. There was a less marked but definite decrease in patients where subtotal thyroidectomy was performed.

The hyperplasia found in the marrow of the hyperthyroid individuals was myeloid in character, and was not reflected in the peripheral blood.

SULFANILAMIDE, Effect of Sulfapyridine and, Upon the Blood Pigments of White Rats, Smith, P. K. Am. J. M. Sc. 200: 183, 1940.

In white rats that had received orally for one month daily doses of sulfanilamide, 760 mg. per kilogram, small, but significant, amounts of methemoglobin and somewhat larger amounts of sulfhemoglobin were found. By comparison with a group of control rats there was a decrease in total hemoglobin of about 10 per cent.

Under similar conditions a daily dose of sulfapyridine, 1,050 mg. per kilogram resulted in a similar amount of methemoglobin without an appreciable amount of sulfhemoglobin. There was no significant reduction in total hemoglobin.

PERNICIOUS ANEMIA, The Erythrocyte Response to Treatment, Riddle, M. C. Am. J. M. Sc. 200: 146, 1940.

From data in 523 patients with pernicious anemia satisfactorily treated, the average weekly increase in the erythrocyte count at the end of two weeks of treatment was found to bear an inverse relationship to the erythrocyte count before treatment.

This relationship is expressed in the equation $I = 0.78 - 0.174 E_0$, where I is the average weekly increase in the erythrocyte count after two weeks of treatment and E_0 is the erythrocyte count before treatment, expressed as millions of erythrocytes per cubic millimeter of blood.

This equation is suggested as a standard for measuring the relative effectiveness of treatment in pernicious anemia.

Observed values equal to or greater than those obtained from this equation indicate adequate treatment. Observed values less than those calculated from the equation indicate inadequate treatment.

The existence of various complicating factors such as concurrent disease, transfusion, or hemorrhage in association with pernicious anemia, invalidate the use of this standard.

HEMOLYTIC ANEMIA, Erythrocyte Morphology in Experimental as Induced by Specific Hemolysin, Tigertt, W. D., Duncan, C. N., and Hight, A. J. Am. J. M. Sc. 200: 173, 1940.

The administration of a specific hemolysin to the donor animal (dog) is followed by a fall in the erythrocyte and hemoglobin levels, proportional to the amount of hemolysin administered and to the titer of the hemolysin.

In the process of cell destruction due to hemolysin (in contrast to the mechanism of hypotonic hemolysis), the erythrocytes approach a spherical form by a diminution in diameter associated with little or no increase in corpuscular volume.

This approach to sphericity is paralleled by proportionately decreased resistance to hypotonic hemolysis.

The length of life in the blood stream of the erythrocytes altered by the hemolysin is inversely proportional to the amount and titer of the hemolysin administered, and survival periods up to one month have been observed.

CEREBROSPINAL FLUID, Calcium and Phosphorus in, in Diabetes Insipidus, Blotner, H. Am. J. M. Sc. 200: 235, 1940.

The concentrations of the calcium, phosphorus, chlorides, and protein were studied in the spinal fluid and blood serum in 10 patients with diabetes insipidus.

The average concentration of the spinal fluid calcium was slightly greater than that of the Ca^{++} content of the serum (1.51 vs. 1.27 mM per liter) in these patients. A series of control patients showed no such difference between their serum Ca^{++} concentrations and cerebrospinal fluid calcium (1.27 vs. 1.24 mM per liter).

Analyses for inorganic phosphate, chloride, and protein showed no significant changes.

Although terse, the book is authoritative, having been written by two outstanding British physicians. It would appear that a text of this nature would also serve admirably for review for advanced medical examinations. The value set on this manual by the profession is attested to by the fact that it has been used successfully for over forty years and has passed through eleven editions. The latest issue is entirely up to date.

In the last section, on clinical diagnosis, the common types of neurologic disease giving rise to vesical abnormalities are discussed and typical examples are given. Many obscure cases of urinary disturbance are dependent on abnormalities of the brain and cord. Disseminated sclerosis ranks next to syphilis in this regard. A certain number of cases are functional. Cystometric methods are emphasized throughout the book. A very good bibliography and index are included.

This book must be read by all who are interested in the urinary bladder, and particularly by urologists and neurologists.

Multiple Human Births*

SEVENTEEN years ago Dr. Newman's book, the *Physiology of Twinning*, was reviewed in these pages. The present volume brings his studies up to date in a popular presentation. It is the first of a series of semipopular books on subjects of science to be published under the sponsorship of the American Association for the Advancement of Science.

Dr. Newman has been most successful in simplifying his presentation for the lay reader and in avoiding technical terminology. Thus, instead of referring to monozygotic twins, he consistently calls them one-egg twins.

With two million twins, triplets, and quadruplets in the United States, there must be ten million persons closely related to twins in one way or another. The volume should, therefore, be of interest to many.

Sections which should be of especial general interest include Siamese Twins, Mirror Imaging, the Dionne Quintuplets, Criminal Twins, and the Life Stories of One-egg Twins who have been reared apart, usually twins who have been left in orphanages. His stories of their chance meetings would be very appropriate for a "Believe It or Not" column.

The Association for the Advancement of Science is to be congratulated for having selected such a thoroughly interesting volume for the inauguration of the series.

The Englishman's Food†

DR. DRUMMOND, who is professor of biochemistry at University College, London, presents almost in novel form an analysis of the foods that the Englishman has eaten since medieval times. The volume represents a tremendous amount of historical research. It possesses a definite medical facet in that the authors analyze the probable food deficiencies in the various centuries, special emphasis being given to the vitamins.

Illustrative of the material covered, Part One deals with Medieval and Tudor England: Production of Food, Quality of Food, Meals of the People, and Their Diet and Health. Deficiency diseases are discussed. The last part, "The Twentieth Century" chapter, deals with dietary problems in the World War. In an Appendix the authors estimate the composition and food value of some of the diets discussed in various parts of the text.

The volume has illustrations gathered from the old English literature.

It may be recommended not only as a reference volume for all who are interested in the history of foods and in nutrition in general but also as a volume of very interesting general reading.

*Multiple Human Births. Twins, Triplets, Quadruplets and Quintuplets. By Horatio Hackett Newman, Ph.D., Sc.D., Professor of Zoology, University of Chicago. Cloth, 214 pages, \$2.50. Doubleday, Doran & Company, Inc., New York, 1940.
†A History of Five Centuries of English Diet by J. C. Drummond, University College, London; and Anne Wilbraham. 574 pages. Square, London, 1939.

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THE INFLUENCE OF CORONARY SCLEROSIS, CHRONIC CONGESTIVE HEART FAILURE, AND MYOCARDIAL FIBROSIS ON CARDIAC HYPERTROPHY*

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ALTHOUGH the nature of the process resulting in cardiac hypertrophy is incompletely understood, the more accepted theory is that hypertrophy usually follows increased work. This view is most convincingly supported by clinical and necropsy studies. Work hypertrophy, according to this view, is similar to the physiologic growth of muscle. The investigators supporting this theory believe that the hypertrophied heart muscle probably received an adequate blood supply and was relatively free of an inflammatory process during the development of the increased muscle weight.

The inability to explain the observed cardiac hypertrophy in some cases even after careful clinical and post-mortem studies has resulted in the concept that cardiac hypertrophy in many instances may not be due solely to increased work. Numerous hypotheses in addition to increased work have been proposed to explain cardiac hypertrophy. Some investigators believe that in some cases decreased blood supply or myocardial inflammation may result in cardiac hypertrophy. This "injury theory" of Horvath¹ has been supported by both experimental and clinical studies.

It has been suggested^{2, 3} that dilatation is the stimulus resulting in cardiac hypertrophy, whether the cause of the dilatation follows increased work, ischemia, or inflammations. Eyster^{4, 5} has made very significant experimental studies regarding the relationship of dilatation to cardiac hypertrophy. He ob-

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served that dilatation always precedes hypertrophy in the experimental animal. He further pointed out that a relatively brief period of dilatation (three to six days) might result in a degree of hypertrophy equal to that seen with experimental aortic valve defects or stenosis. In further studies Eyster produced temporary cardiac dilatation by increasing the blood volume in rabbits that resulted in increased heart weights. Herrmann and Decherd⁶ were unable to confirm the latter experiment in a recent study.

Davis and Blumgart,² in the study of a small number of cases, offer further evidence to support the "injury" concept; they found that those patients presenting congestive failure had hearts weighing more than those without failure. The hearts in their study with severe coronary sclerosis were heavier than those with only a mild degree of coronary sclerosis.

Cardiac hypertrophy has not been noted in experimental cardiac infarction in dogs (Sutton and Davis⁷) nor has it been observed in serial roentgenologic examinations in individuals thought to have a normal-sized heart at the time they experienced a coronary thrombosis (Horine and Weiss⁸). Gross⁹ failed to observe any constant relation between coronary sclerosis and myocardial fibrosis. Miller and Weiss¹⁰ believed that in case of gradual occlusion adequate collateral circulation develops which may forestall hypertrophy. Clawson¹¹ has emphasized that ischemia due to narrowing of the coronary orifices in syphilitic aortitis failed to produce hypertrophy when unassociated with valvular insufficiency. He further points out that any hypertrophy observed in those hearts having coronary sclerosis is more likely to be due to an associated hypertension found in a large percentage of cases than to the coronary sclerosis alone. In a careful study of 420 post-mortem records, Bell and Clawson¹² found coronary sclerosis more frequent in hypertensive than in nonhypertensive individuals and believe there is little evidence to support the concept that hypertrophy is found in an ischemic muscle. Bean¹³ noted hearts with infarcts were usually enlarged when associated with hypertension.

Assuming that ischemia due to coronary sclerosis, congestive failure, or myocardial damage might produce cardiac hypertrophy, it would seem probable that with a severe degree of coronary sclerosis, congestive heart failure, or myocardial fibrosis, the heart would become unusually large; it would be relatively small with only a mild degree of sclerosis or failure. This study was conducted to investigate this point of view.

MATERIAL

The reports of 1,645 autopsies performed at Ancker Hospital were studied. In this group 411 patients were found to have some degree of coronary sclerosis. The post-mortem examinations were done under the direction of Dr. John F. Noble or by myself. It is advantageous to secure the material for a study of this type in which the descriptions of the arteries conform to a uniform pattern, so that the investigator might judge with some accuracy the degree of sclerosis indicated. In order to make an adequate study of post-mortem records one must be familiar with the terminology used by the observer in recording the autopsy. The exact inference of the terms used to describe the coronary

It should be remembered that in the routine post-mortem examinations one does not dissect the entire heart muscle or secure sections from sufficiently numerous areas to be entirely certain that fibrotic patches are not present. In most laboratories the pathologist examines the most superficial portion of the septum and several cut surfaces of the left ventricle. A careful study of the entire heart often reveals surprisingly numerous areas of fibrosis.

Heart Failure.—The presence or absence of heart failure, as well as the degree of failure, was recorded. The more obvious signs of heart failure, such as edema, ascites, and hydrothorax, furnished a great deal of information, but of equal importance was careful gross and microscopic examination of the lungs and liver. Congestion of the lungs was noted to be somewhat proportional to the number of macrophages in the alveoli containing blood pigment. Similarly, the congestion about the central veins of the liver was considered an adequate index of heart failure. A correlation of the microscopic findings in the lungs and liver with the gross observations at the post-mortem examination enables one to classify the case as to the absence or degree of heart failure. Consequently it was graded as absent, mild, or severe.

OTHER CAUSES OF INCREASED HEART WEIGHTS

Coronary sclerosis was noted to be present in hypertrophied hearts whose increased weights were thought to be due to valve defects, adherent pericardium, hypertension, etc. In order to study the effect of coronary sclerosis alone on the heart weight, it was necessary to tabulate the average weight of hearts with coronary sclerosis alone in comparison to the average weight of hearts with coronary disease plus hypertension, adherent pericardium, or valve defects. Should coronary sclerosis alone fail to increase the weight of an observed group of hearts then one would likely consider its effect minimal in other hypertrophied hearts in which coronary sclerosis coexists with hypertension, valve defect, or adherent pericardium.

ISCHEMIA

The effect of ischemia on cardiac weight can be studied by examination of the data in Charts 1, 2, and 3. In order to study the theory that "myocardial injury" due to ischemia might result in dilatation and subsequent hypertrophy, the cases were classified as to their various degrees of coronary sclerosis, ranging from grades I to IV. It was assumed that the hearts with severe sclerosis would have vessels with very small lumina, and hence the muscle would be quite ischemic. The "damage" due to the ischemia or previous infarcts would presumably produce hypertrophy according to the concepts proposed by Horvath.¹ It would then be logical to expect the hearts with severe coronary sclerosis to weigh more than those with minimal vessel changes.

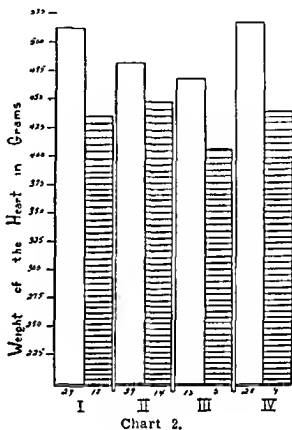
Chart 1 is a record of the entire group studied, representing 305 males and 106 females with varying degrees of coronary sclerosis arbitrarily divided in grades I to IV sclerosis, depending on the degree of narrowing of the vessels. In these 411 patients coronary sclerosis is associated with all other possible causes of hypertrophy. In the 52 most ischemic male hearts (grade IV) the average weight is seen to be 365 Gm. while the hearts with grade III sclerosis

weigh 408 Gm. Grade III cases, which should have less blood supply than those with grade II sclerosis, have an average weight of 429 Gm. A total of 119 male hearts with grade I sclerosis weigh 415 Gm. These hearts are heavier than the apparently more ischemic group with grade III sclerosis. Examination of the female patients tabulated in Chart 1 reveals those with grade IV coronary sclerosis have an average weight (367 Gm.) greater than those with grade I sclerosis (339 Gm.), but less than those with grade II and III sclerosis (386 and 382 Gm., respectively).



Chart 1.—Coronary sclerosis. All cases.

Chart 2.—Heart weight with coronary sclerosis and hypertension. See Chart 1 for key.



In Chart 1 coronary sclerosis is present with hypertension, adherent pericardium, valve defects, etc. It is apparent, however, that the weight of the heart is not related to the degree of observed coronary sclerosis and probable ischemia present. From these data the evidence points to the fact that the heart weight is dependent on factors other than coronary disease. The average weights of the hearts in various grades of sclerosis would seem to be a matter of chance selection.

Several investigators have noted that hearts with severe coronary sclerosis and cardiac hypertrophy usually have an associated hypertension. It is also striking that a group of hearts with coexisting hypertension and coronary disease (Chart 2) have an average weight greater than that of an unselected group of hearts (Chart 1), and that this weight is far in excess of that of a group of hearts with coronary sclerosis alone (Chart 3). The latter group in this study fail to show any evidence of an average increased weight due to coronary sclerosis alone. A comparison of these charts indicates that a heart with hypertension will likely be hypertrophied with or without coronary sclerosis, while those hearts with coronary sclerosis alone will likely be approximately normal in weight. In order to re-emphasize the causal relationship of coronary disease to hypertension 157

hearts are tabulated in Chart 2. In this group there is some clinical or anatomic evidence of hypertension in addition to the sclerotic changes of the coronary vessels as observed in the post-mortem specimens. This study verifies Clawson's¹¹ opinion that there is a very close relationship between hypertension and coronary sclerosis.

HYPERTENSION AND CORONARY SCLEROSIS

Chart 2 is a study of those cases in which the heart weights might be due to either hypertension or coronary sclerosis, or both. From a study of this group the hearts (male) with grade IV sclerosis have an average weight of 523 Gm., which is somewhat more than those with grades III and II sclerosis (469 and 486 Gm., respectively). The average weight of the 29 hearts with grade I coronary sclerosis and hypertension is 514 Gm., which is much greater than the more ischemic hearts with grades II and III sclerosis.

There are 46 females tabulated in this group. In this series, those hearts with grade II sclerosis weigh 451 Gm., while those with grade IV sclerosis weigh 441 Gm. Hearts with grade III have an average weight of 432 Gm. as compared to an average of 438 Gm. with grade I coronary disease. This would also indicate that the heart weight is unrelated to the muscle ischemia or "injury." It would be assumed that the heart weights are influenced in this group by the presence of hypertension, since all grades of coronary sclerosis show a variable degree of hypertrophy. Ischemia apparently has little influence on the weights of hearts in the presence of an existing hypertension.

Coronary Sclerosis Alone.—A survey of the 159 male hearts reveals the average weight to be less than 372 Gm., which would not be considered a hypertrophied heart in comparison to average adult male hearts in the material examined in this department. It is also striking that the average weight of the hearts in this group is 50 to 75 Gm. less than those in Chart 2 that have an associated hypertension. The average weight of the hearts studied in Chart 3 is also considerably less than those in Chart 1 in which the heart weight might be due to coronary sclerosis plus hypertension, adherent pericardium, or valve defects. A comparison of these charts confirms Clawson's opinion that hypertension is the likely cause of the increased heart weight in those cases with both hypertension and coronary sclerosis.

A total of 208 hearts are tabulated in Chart 3 in which coronary sclerosis is the only possible cause of the increased heart weight. Additional factors that might produce hypertrophy were excluded after careful examination of clinical records and post-mortem studies. In this group the effect of ischemia on cardiac weight can be correctly analyzed, since no other factor that might produce hypertrophy is present.

One would expect hearts with the more ischemic muscle to be the largest if coronary sclerosis produced hypertrophy. The average weight of 23 male hearts with grade IV sclerosis is 357 Gm., while the weight of the less ischemic hearts (grade III sclerosis) is 371 Gm. The 70 hearts with grade I sclerosis have an average weight of 349 Gm., while those with grade II sclerosis have an average weight of 334 Gm.

The average weight of the female hearts tabulated in Chart 3 is not proportional to the degree of coronary sclerosis present, nor are the hearts larger than the normal hearts encountered in this department. The heaviest hearts are found to have an average weight of only 371 Gm. (grade III sclerosis). These data fail to confirm the theory that ischemia, or "cardiac injury," produces hypertrophy, since the hearts with mild coronary disease are approximately the weight of those with severe coronary sclerosis.

Davis and Blumgart have presented evidence to show that chronic congestive heart failure may in itself produce hypertrophy. The view is divergent to the common concept that congestive failure is seen in a previously hypertrophied heart.

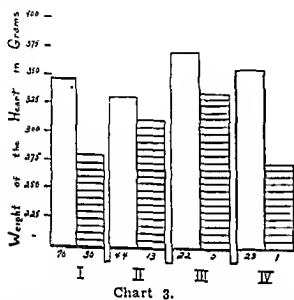


Chart 3.—Heart weights in cases of coronary sclerosis without hypertension. See Chart 1 for key.

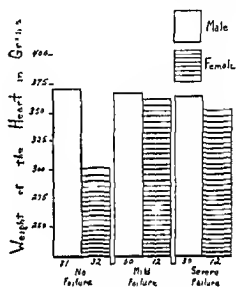


Chart 4.

Chart 4.—The relation of chronic congestive heart failure to heart weight

CHRONIC CONGESTIVE FAILURE

Chart 4 is a record of 222 patients showing some degree of coronary sclerosis associated with some degree of chronic congestive heart failure in some cases and no failure in others. Since in the preceding charts coronary sclerosis was found to have little influence on the heart weight, congestive failure would be the only factor influencing the weight in these cases. Hypertension and the other usual causes of cardiac hypertrophy are not present in the cases studied in Chart 4. Should one assume that chronic congestive failure produces cardiac hypertrophy, then the hearts with severe failure should have an average weight greater than the weight of those with mild failure, while both should weigh more than the group with no gross or microscopic evidence of heart failure.

Examination of the data shows the average weight of all the hearts to be from 50 to 75 Gm. less than those usually considered in the hypertension group in this department. The weights of the entire group approximate the weights of the hearts due to coronary sclerosis alone (Chart 3). The patients with severe heart failure weigh approximately the same as those with mild heart failure and those with no evidence of heart failure. There is little convincing evidence from this study that chronic congestive failure might produce cardiac hypertrophy.

MYOCARDIAL FIBROSIS

From an anatomic viewpoint one might observe the influence of "myocardial injury" by noting the effect of myocardial fibrosis on the heart weights. Fifty-seven patients have been classified in Chart 5 as to their degree of myocardial fibrosis and heart weights—all other possible causes of increased heart weight have been excluded after a careful study of the clinical records and examination of both the gross and microscopic specimens. It is surprising that male hearts with grade IV fibrosis weigh less than those with grade I. The hearts with grade III fibrosis also weigh less than those with grade II fibrosis. Consideration of the data in regard to the male specimens would indicate that in the presence of myocardial damage the average weight is about equal to that of a normal heart. In the same chart the average weight of the female specimens increased as the myocardial fibrosis became more severe. The material presented adds no support to the theory that myocardial injury will produce cardiac hypertrophy.



Chart 5.—Relation of myocardial fibrosis to hypertrophy.

In this study only 25 persons were found to have thrombosed coronary arteries. Since the majority of these were found to have gross or microscopic evidence of hypertension, the number is too small to reach any conclusion in regard to the relation of thrombi and cardiac hypertrophy.

The data in this material were examined by statistical methods. A comparison of the average weights was shown to be sufficiently accurate in a study of this kind.

DISCUSSION

A total of 411 cases of coronary sclerosis were studied. An attempt was made to correlate the heart weight with the degree of coronary sclerosis, "myocardial damage," and congestive failure present. Practically all investigators found it difficult to explain satisfactorily the observed increased heart weight in a small percentage of the specimens seen at post mortem. For a few of this group, several investigators have suggested that an injured myocardium may be

the stimulus that results in subsequent hypertrophy. An obvious cause of such injury would likely be decreased blood supply due to coronary sclerosis. This theory has received support by competent observers who have frequently noted severe coronary sclerosis as the only obvious explanation of the increased heart weight. In addition to the presence of disturbed nutrition, Davis and Blumgart² have presented some evidence to show that chronic congestive failure may be associated with hypertrophied hearts.

In contrast to these views other workers have failed to observe any evidence of increased heart weight due to coronary disease that might produce ischemia or myocardial injury. The usual belief is that adequate blood supply is necessary for any muscle hypertrophy. These investigators also found a close correlation between cardiac hypertrophy and hypertension.

To study the effect of ischemia on hypertrophy the hearts were separated into various grades of coronary sclerosis, and the average heart weight was determined in the various groups. No correlation was noted between the degree of sclerosis present and the heart weight at post mortem. It was found that hypertension was the usual cause of the increased weight and that those patients with coronary sclerosis alone as the explanation of the heart weight could not be placed in the hypertrophied group.

Eyster^{4, 5} presented experimental hypertrophy in dogs by producing dilatation of the ventricle by means of an experimental valve defect or aortic stenosis. Hypertrophy followed the stretching of the muscle fibers. The hypertrophy was considered a reaction to the "myocardial damage." Similarly, others have reasoned that the increased diastolic volume in a failing heart may result in stretching the muscle fibers and thereby result in hypertrophy.

The effect of chronic congestive failure on heart weight was studied by comparing the average weight of the cases with no heart failure, mild failure, and severe failure. After eliminating all other factors, those patients without heart failure were found to be equal in weight to those with severe failure. There was little evidence that the presence of heart failure would influence the heart weight.

In order to approach the injury thesis from another viewpoint the weights of the hearts with various degrees of myocardial fibrosis were compared. It was assumed that the amount of fibrosis present was some index of the myocardial injury. If the injury thesis is correct, it is reasonable to assume that the hearts with severe fibrosis would be larger than those with mild degree of fibrosis. From this study there is no correlation between the degree of fibrosis and the observed heart weight.

CONCLUSIONS

1. The average weight of 411 hearts and the degree of coronary sclerosis were compared.
2. There was no correlation between the degree of coronary sclerosis and the heart weight.
3. Hypertension was the usual explanation of the increased heart weight, and a close relationship between coronary sclerosis and hypertension was noted.

4. The hearts with no heart failure were found to weigh approximately the same as those with chronic congestive heart failure.

5. No evidence was found to support the concept that myocardial fibrosis may produce cardiac hypertrophy.

REFERENCES

1. Horvath, A.: Über die Hypertrophie des Herzens. Aus den Vorlesungen über Allgemeine Pathologie; Mit einem Vorwort von A. Weischelbaum, Wien and Leipzig, 1897, W. Braumüller.
2. Davis, D., and Blumgart, H.: *Ann. Int. Med.* 11: 1024, 1937.
3. Fishberg, A. M.: *Heart Failure*, Philadelphia, 1937, Lea & Febiger.
4. Eyster, J. A. E.: *Cardiac Dilatation and Hypertrophy*, Tr. A. Am. Physicians 42: 15, 1927.
5. Eyster, J. A. E., Meek, W. J., and Hodges, P. J.: *Cardiac Changes Subsequent to Experimental Aortic Lesions*, *Arch. Int. Med.* 39: 536, 1927.
6. Herrmann, G., and Decherd, G.: *Some Studies in the Mechanism of Cardiac Hypertrophy*, *Ann. Int. Med.* 13: 794, 1939.
7. Sutton, D., and Davis, M.: *Effects of Exercise on Experimental Cardiac Infarction*, *Arch. Int. Med.* 48: 1118, 1931.
8. Horine, E. P., and Weiss, M. M.: *Coronary Thrombosis and Its Effect on Size of Heart*, *Am. J. M. Sc.* 189: 858, 1935.
9. Gross, H., and Spark, C.: *Coronary and Extra Coronary Factors in Hypertensive Heart Failure*, *Am. Heart J.* 14: 161, 1937.
10. Miller, H. R., and Weiss, M. M.: *Disease of the Coronary Arteries; Its Occurrence Without Gross Cardiac Hypertrophy*, *Arch. Int. Med.* 42: 74, 1928.
11. Clawson, B. J.: *Coronary Sclerosis*, *Am. Heart J.* 17: 387, 1939.
12. Bell, E. T., and Clawson, B. J.: *Primary (Essential) Hypertension; Study of 420 Cases*, *Arch. Path.* 5: 939, 1928.
13. Bean, W. B.: *Infarction of the Heart; Clinical Course and Morphological Findings*, *Ann. Int. Med.* 12: 71, 1938.
14. Smith, H. L.: *The Relation of the Weight of the Heart to the Weight of the Body and of the Weight of the Heart to Size*, *Am. Heart J.* 4: 79, 1928.

DISSECTING ANEURYSM OF THE AORTA*

A SUMMARY OF CLINICAL SYNDROME AND PATHOLOGY TOGETHER WITH REPORT OF A CASE SHOWING UNUSUAL PATHOLOGIC FEATURES

FREDERICK W. NIEHAUS, M.D., AND WILLIS D. WRIGHT, M.D., OMAHA, NEB.

DURING the last decade many cases of dissecting aneurysm have been reported. Several comprehensive reviews^{1, 4, 17} have appeared in the literature describing the pathology^{10, 16, 17} and the clinical features.^{2, 3, 7} With this interest in the subject the number of cases diagnosed ante mortem, and corroborated by autopsy, has greatly increased.

These cases have been so well described and carefully studied pathologically and clinically that it seems superfluous to record another case, unless new features have been observed. Our case herein described presents some unusual features. Deep longitudinal grooves of the ascending and the transverse arch of the aorta, causing narrowing of the isthmus, are mentioned only in one other case, which was reported by Klotz and Simpson.⁶ Our case contributes clinically to the evaluation of the electrocardiogram in dissecting aneurysm.

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REPORT OF CASE

History.—W. Mc., a negro porter, aged 49 years, was seized by a sudden severe pain on November 2, 1937, at 7 P.M., while standing on a depot platform waiting for passengers to board a train. At this stop no passengers left or entered the train, so that he had no baggage to carry. The only exertion immediately prior to the onset of the pain consisted of carrying a small stool such as is usually placed at the foot of passenger car steps.

Pain.—The onset was very sudden and extremely severe. He was prostrated and "broke out in a cold sweat." He was put to bed in a berth.

At the onset the pain radiated to his neck and jaws, then seemed to penetrate to his back in the lower thoracic region, then radiated downward to the lumbar region and the backs of his thighs as far as his knees. In about an hour the pain localized in his upper abdomen. At 11 P.M. he was met at the train by one of us (W. D. W.) and immediately sent to a hospital. The pain at this time was localized in the epigastrium and the lower sternal region, radiating to the interscapular region and along both costal margins, being more marked toward the right side. The pain persisted at this site until death occurred, twenty-one hours after the onset. The severity remained intense in spite of the liberal use of morphine.



Fig 1.—Electrocardiogram showing inverted T_1 and T_2 and depressed S-T segment and absent Q in Lead IV.

On arrival at the hospital the pulse rate was sixty per minute, the blood pressure was 140 systolic and 100 diastolic. He had not vomited, but was moderately distended. The epigastric pain was intense. There was marked tenderness with some muscle spasticity of the epigastrium and the right upper abdominal quadrant, stimulating upper abdominal disease. The next morning at 8 A.M. the pain remained localized in the epigastrium, still very severe, except when ameliorated by opiates.

Examination.—Examination showed a robust, obese negro, weighing about 200 pounds, appearing to be critically ill and in agony. His face was suffused and veins of his neck were moderately distended. There was only a slight respiratory difficulty. The pupils reacted to light and were equal in size. The lungs showed no change of density or râles anteriorly or laterally. On account of his critical condition it was not deemed advisable to turn him.

The heart showed the apex in the anterior axillary line in the fifth interspace. There was an extension of dullness to the left in the third interspace. The rhythm was regular. The pulse rate was 100 per minute. There was a definite protodiastolic gallop rhythm, and a diastolic murmur at the aortic area and along left sternal border. The epigastrium and the right upper abdominal quadrant were tender and moderately spastic. The blood pressure was systolic 140 and diastolic 100.

Urinalysis: The urine was acid and had a specific gravity of 1.037. It showed albumin 2+, and was negative to sugar. Microscopic examination revealed numerous pus cells.

Blood: The hemoglobin was 15.6 Gm., erythrocytes were 4,700,000 and leucocytes 9,000. The differential count showed segmented cells 51 per cent, staff cells 14 per cent, lymphocytes 30 per cent, and monocytes 5 per cent. The icteric index was 6. The sedimentation rate was 3 mm. in one hour. The blood Wassermann reaction was negative.

An electrocardiogram (Fig. 1) taken at 8:30 A.M. on November 3, 1937, showed evidence of coronary thrombosis. The clinical picture, while atypical, indicated a circulatory disorder. Together these seemed to justify the diagnosis of coronary occlusion. The patient's condition remained unchanged until 4 P.M. (twenty-one hours after onset) when he suddenly died while taking a drink of water.

The following findings were available from the company's records of periodic examinations made by Dr. L. Chaffin of Los Angeles.

Feb. 20, 1923: Weight 175½ pounds. Blood pressure 122/85, Wassermann 4+. Urine showed a trace of albumin.

Feb. 15, 1924: Weight 201½ pounds. Blood pressure 130/80.

March 15, 1925: Weight 206½ pounds. Blood pressure 135/78. Faint trace of albumin was present in urine.

Jan. 12, 1932: Weight 220 pounds. Blood pressure 230/140. Urine negative.

Jan. 15, 1933: Weight 215 pounds. Blood pressure 226/124. Urine negative. Left side of heart enlarged.

Jan. 9, 1934: Weight 215 pounds. Blood pressure 210/124. Urine negative. Teeth carious. Left side of heart enlarged. No murmurs. Varicose ulcer on left leg.

Aug. 22, 1933 to Feb. 2, 1934: Disabled on account of varicose ulcer on left leg.

Jan. 28, 1935: Weight 215 pounds. Blood pressure 200/120. Varicose ulcer healed. Urine negative.

Jan. 13, 1936: Weight 210 pounds. Blood pressure 200/130. Urine; specific gravity 1.020, albumin 2+, sugar negative. Varicose veins on left leg.

Autopsy.—The autopsy performed by Dr. A. S. Rubnitz showed the following significant findings: The pericardial sac was widely distended, containing about 1,000 c.c. of clotted blood. After removing this, a large subpericardial hematoma enveloped the origin of the aorta, the pulmonary artery, and the right auricle. The aorta showed irregular triangular rupture (Fig. 2) 1.5 cm. in length, about 2.5 cm. above the aortic valve ring. This was located on the right lateral surface, slightly anteriorly, and extended through the intima and about four-fifths of the media. From the site of this rupture the blood had burrowed toward the heart, involving almost all the circumference of the aorta, and thence extended subpericardially over the surface of right auricle as described. The site of rupture through the visceral pericardium could not be demonstrated. The intramural hematoma extended distally from the site of the rupture along the ascending, the transverse, and the descending arch of the aorta, terminating at the level of the fourth thoracic vertebra. The extent of the circumference dissected varied from about one-half at the site of the rupture to one-fourth at its termination. The aorta showed minimal arteriosclerotic changes. The ascending arch shows small slightly elevated yellowish plaques, the largest being not over 3 mm. thick. There was no gross evidence of mesaortitis. At the site of rupture the aortic wall showed no further defects. The torn edge showed a normal thickness of media and a minimal intimal thickening. At the juncture of the ascending and the transverse arch of the aorta there were four or five peculiar longitudinal grooves (Fig. 3), measuring 1.5 cm. in diameter; these reduced the size of the lumen of the aorta considerably. Beyond this the arch widened to what appeared to be a normal diameter. The size of the intimal arteriosclerotic plaques increased in size (0.5 cm.), and were more numerous in the lower thoracic and the abdominal aorta. However, throughout the entire length of the aorta there were no atheromatous ulcers or calcified plaques.

The left ventricle of the heart was moderately hypertrophied. The right side of the heart was dilated. There was no thickening, retraction, or sclerosis of any of the heart valves. In view of the fact that syphilis has been considered as an etiologic factor in rupture of the aorta, it is particularly noteworthy that in this case the aortic ring was not widened, the aortic cusps were soft, thin, and free from fibrotic changes or retractions.



Fig. 2.—Photograph showing site of aortic rupture and folds in aorta.

The coronary arteries presented no narrowing of orifices. The branches were opened to their terminations. There was no thrombus or narrowing of the lumen. In a few places there was slight intimal thickening appearing as subintimal yellow plaques, but no atheromatous ulcers or areas of calcification were present. There was a moderate tortuosity of the larger branches of the coronary arteries on the surface of the myocardium. Lungs showed marked congestion. Kidneys showed few small cysts. The other organs showed no significant changes.

Dr. H. E. Eggers, Professor of Pathology at University of Nebraska, reported the following microscopic examination of the aortic wall: Sections were cut both through the involved portion and what appeared to be an intact portion of the vessel. The latter section showed no evident change, whereas the former revealed a zone of necrosis of the media, extending for some distance as a narrow band, but widening centrally, and disrupted in the widest part. To the extent that the vasa vasorum could be distinguished, these were intact, even in close proximity to the diseased area. None could be found in the necrotic portions proper. Hemorrhage was noted in the gap where disruption of the wall occurred.

PATHOLOGY OF DISSECTING ANEURYSMS OF AORTA

Recent authors⁹ generally do not consider syphilis as an etiologic factor. Klotz and Simpson⁶ logically state that a granulomatous inflammatory process like syphilis would tend to weld the lamellae more closely together, so that "the wall would split less easily into its anatomic layers." Our patient showed a negative Wassermann at the time of this attack, the aortic wall showed no gross or microscopic evidence of syphilis, and the aortic cusps were entirely normal. In spite of the fact that this patient had a record of a four-plus Wassermann reaction of the blood in 1923, there was no evidence of a syphilitic process in the aorta at the time of autopsy.

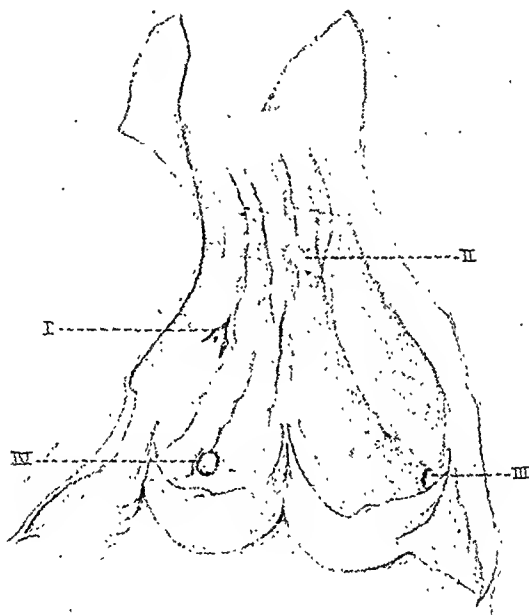


Fig. 3.—Drawing showing: I, Rupture of aortic wall; II, longitudinal grooves which narrowed the lumen of the aorta; III, orifice of right coronary artery; and IV, orifice of left coronary artery.

Changes in the media mentioned by Gsell,¹⁸ described by Erdheim¹⁶ as an idiopathic cystic medial necrosis, and elaborated by Moritz,¹⁷ are accepted as the characteristic lesion. This is described as "small areas of necrosis and peculiar hyalinized vacuoles in the media," showing "no inflammatory or reparative process." These form "peculiar cystic spaces between elastic fibers." The vasa vasorum may rupture into these spaces, forming a hematoma which splits the layers of the media. These may rupture the intima secondarily;¹⁰ probably primary rupture through the intima occurs more frequently. Tears nearly always occur in the ascending arch of the aorta. Dissection usually occurs between middle and outer thirds of the wall, and involves one-third to one-half of the circumference of the aorta. This dissection may extend toward the heart, entering the pericardial sac and surrounding part of the heart with a hematoma; it may interfere with the coronary circulation, and finally terminate by rupture into the pericardial sac.³ Our case is an example of this course,

which explains some of the symptoms and the electrocardiographic findings. Others³ have described rupture into the mediastinum, pleural cavity, or abdomen. This channel may re-enter the aorta forming a "double barreled" aorta.

The dissection produces decided effects on the vessels originating directly from the aorta³ and may result in (a) severance as in the case of the intercostal arteries, (b) partial or rarely complete obstruction by the hematoma, (c) extension of the dissection along the larger branches such as the renal vessels, or (d) disruption of the periarterial sympathetic plexus resulting in a spontaneous periarterial sympathectomy.⁹ These would produce bizarre vascular and neurologic symptoms and signs, such as changes in the pulse, paresthesia, hyperesthesia, anesthesia, or paralysis. Death occurs by rupture of the sac of the hematoma into a cavity, and very rarely by cardiac failure.

TABLE I

FEATURES	DISSECTING ANEURYSM OF AORTA	CORONARY THROMBOSIS
Onset	(1) Sudden (2) With exertion	(1) Sudden (2) Usually at rest
Pain		
(a) Character	Extremely severe, tearing	Severe
(b) Radiation	Widespread, chest, abdomen back thighs; arms rarely	Chest, neck, arms usually
(c) Duration	Minutes to days	Hours to days
Syncope	Frequent	Rare
Shock	Frequent with normal, or elevated blood pressure	Frequent with low blood pressure
Fever	Moderate	Low
Leucocytosis	Moderate	Low
Pulse	Often unequal, may be decreased or increased	Usually equal
Hypertension	Usually present or history of such; may vary in two arms	With attack usually low, may or may not be history of such; same both arms
Pericarditis	None	Pericardial friction rub present at times
Diastolic murmur	Important sign	Not related to condition
Hematuria	May be present	None
X ray	Deformity of aorta, rapidly increasing in size	Probable changes of heart size and shape
Electrocardiogram	If negative, important finding ¹⁴	Usually presents typical electrocardiogram
Neurologic	Bizarre, paresthesia, anesthesia, hyperesthesia, paralysis from circulatory interference	Usually absent; when present due to emboli
Venous pressure	Extremely high if cardiac tamponage	High with congestive failure

In our case the peculiar longitudinal grooves (Fig. 3) involving the distal part of the ascending and the proximal part of the transverse arch of the aorta were conspicuously present. This reduced the size (1.5 cm.) of the lumen considerably, and thereby may have caused an increase of pressure with systole in the first part of the ascending aorta. These grooves have hitherto been mentioned in only one other case, which was reported by Klotz and Simpson.⁶ The aortic wall was not thickened between these grooves. They give the impression of being formed by a decrease of substance in the aortic wall, as if the grooves were the marks on the intimal surface of subjacent longitudinal vacuoles. Microscopic examination of the wall of the aorta by Eggers showed the typical medial changes described by Erdheim.¹⁶

CLINICAL MANIFESTATIONS

The symptoms and signs of a dissecting aneurysm have been clarified by extensive and thorough study of the pathology, so that a clinical syndrome can be synthesized.

1. The onset is usually sudden and violent.³
2. The pain is extremely severe and agonizing,³ described as having a tearing quality, accompanied by a sense of annihilation. A few persons are reported who suffered no pain during the illness.^{7, 11}
3. The duration of the pain is from minutes to days.
4. The radiation of the pain is widespread and bizarre. It usually begins in the anterior part of the thorax, spreading to the neck, to the back in the thoracic, the lumbar, or the sacral regions, to the thighs or to the upper part of the abdomen. Radiation of pain to the arms rarely occurs, but when present, it is felt on the outer surface of the arms.⁷
5. Syncope¹ and shock occur in nearly all cases. Shock in this condition, is frequently not accompanied by the usual drop in the blood pressure. Nausea and vomiting are common.
6. Fever and leucocytosis of a moderately high degree are present in most cases.
7. Circulatory phenomena.⁷
 - (a) The peripheral pulses may be altered. The dissection of the layers of the aorta and resultant hematoma may partially obstruct the branches leaving the aorta, resulting in a diminution of pulse tension and volume in this vessel. The degree varies from a slight decrease to almost complete obliteration. In the case of an extremity this results in varying degrees of numbness, coldness, and weakness. The pulsation may, however, be increased, if the dissection extends onto the branching vessel, thereby producing a spontaneous periarterial sympathectomy.⁷ This mechanism may have an influence in maintaining a normal pressure in the presence of systemic signs of shock. In the case of the renal vessels and kidneys, this circulatory disturbance may cause hematuria.¹¹
 - (b) Hypertension is usually present, although a number of cases have been recorded with the presence and the history of a normal blood pressure.^{9, 10} There may be an inequality of pressure in the arms.
 - (c) A diastolic aortic murmur⁷⁻⁹ has frequently been reported. Hamburger and Ferris record the development of such a murmur during their observation of a case of dissecting aneurysm. This murmur was distinctly evident in our patient. Such a finding is not accompanied by the peripheral signs of aortic regurgitation.
 - (d) A hematoma in the mediastinum or blood in the pericardium may produce signs of cardiac tamponade, with widely distended cervical veins and a very high venous pressure, as was present in our case. At autopsy this was accounted for by the presence of a large amount of blood in the pericardial sac.
 - (e) Roentgenologic signs²⁰ consist of a deformity of the supracardiac shadow, or of the aorta, which may rapidly increase in size. If the shadow pulsates, it is a particularly reliable sign.

- (f) The electrocardiogram¹ is frequently not modified, but cardiac dissection of the aneurysm may disturb the coronary circulation sufficiently to produce an electrocardiogram, indicating a coronary obstruction.¹³ This was exemplified in our case (Fig. 19).

NEUROLOGIC SIGNS

Hemiplegia may be caused by dissection extending cephalad along the left carotid artery, thereby obstructing this vessel.

Bizarre neurologic symptoms, such as paresthesia, anesthesia, hyperesthesia, or even paralysis, may result from circulatory interference to the extremities or other parts. As previously mentioned, the intercostal vessels may be torn from the aorta. Even larger vessels may be torn from their site of origin or the dissection extending along their walls with hematoma formation may more or less obstruct their lumina. Increased heat of an extremity may be produced by spontaneous periarterial sympathectomy as already mentioned.

DIFFERENTIAL DIAGNOSIS

The onset and course of a dissecting aneurysm of the aorta and coronary thrombosis present many common features. An attempt at differentiation is shown in Table I.

SUMMARY

1. A case of dissecting aneurysm of the aorta, showing peculiar longitudinal grooves of the isthmus, is presented.
2. A synthesis of a clinical syndrome is attempted from the pathology, symptoms, and signs.

REFERENCES

1. Shennan, T.: *Dissecting Aneurysms*, London, 1934, H. M. Stationery Office.
2. Peery, T. M.: *Dissecting Aneurysms of the Aorta*, *Am. Heart J.* 12: 650, 1936.
3. McGeachy, T. E., and Paullin, J. E.: *Dissecting Aneurysm of the Aorta*, *J. A. M. A.* 108: 1690, 1937.
4. Glendy, R. E., Castleman, B., and White, P. D.: *Dissecting Aneurysm of Aorta, A Clinical and Anatomical Analysis of Nineteen Cases (Thirteen Acute) With Notes on Differential Diagnosis*, *Am. Heart J.* 13: 129, 1937.
5. Roessler, H., Gifford, U. G., and Betts, W.: *Dissecting Aneurysm of the Aorta, With Description of a Sign Heretofore Not Mentioned*, *Am. Heart J.* 13: 426, 1937.
6. Klotz, O., and Simpson, W.: *Spontaneous Rupture of the Aorta*, *Am. J. M. Sc.* 184: 455, 1932.
7. Hamburger, M., and Ferris, E. B.: *Dissecting Aneurysm*, *Am. Heart J.* 16: 1, 1938.
8. Resnick, W., and Keefer, C.: *Dissecting Aneurysm With Signs of Aortic Insufficiency*, *J. A. M. A.* 85: 422, 1925.
9. Hamman, L., and Apperly, F.: *Spontaneous Rupture of Aorta With Aortic Insufficiency*, *Internat. Clin.* 4: 251, 1933.
10. Schattenberg, H. J., and Ziskind, J.: *Dissecting Aneurysm of Aorta*, *J. LAB. & CLIN. MED.* 24: 264, 1938.
11. Rogers, H.: *Dissecting Aneurysm of Aorta*, *Am. Heart J.* 18: 67, 1939.
12. Roberts, J. T.: *Medionecrosis Aortae Idiopathica Cystica*, *Am. Heart J.* 18: 188, 1939.
13. Hardway, R. M., and Green, M. M.: *Intrapericardial Rupture of Aorta*, *Am. Heart J.* 10: 384, 1935.
14. Norris, J. C.: *Transverse Rupture of Aorta*, *South. M. J.* 27: 733, 1934.
15. Arenberg, H.: *Spontaneous Rupture of Aorta*, *Am. Heart J.* 8: 217, 1932.
16. Erdheim, J.: *Medionecrosis Aortae Idiopathica Cystica*, *Virchows Arch. f. path. Anat.* 276: 187, 1930.
17. Moritz, A. R.: *Medionecrosis Aortae Idiopathica Cystica*, *Am. J. Path.* 8: 717, 1932.
18. Gsell, O.: *Virchows Arch. f. path. Anat.* 270: 1, 1928.
19. Henke, F., and Lubarsch, O.: *Handb. d. spez. path. Anat. u. Histol.* 11: 673, 1928.
20. Wood, E. C., and others: *Dissecting Aneurysm of the Aorta With Special Reference to Its Roentgenographic Features*, *Am. J. Roentgenol.* 28: 437, 1932.

SULFAPYRIDINE OVERDOSAGE—ANTIDOTAL ACTION OF HYPNOTICS IN ANIMALS*

WITH A NOTE ON SULFATHIAZOLE

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SULFAPYRIDINE

THE first reports on the toxicity of sulfapyridine in experimental animals by Wien¹ seem to indicate a lower toxicity of this drug as compared with sulfanilamide. However, subsequent studies, particularly by Marshall, Bratton, and Litchfield,² have shown that the incomplete absorption due to the low solubility of sulfapyridine is responsible for the alleged lower toxicity. If this factor is taken into consideration, sulfapyridine is actually somewhat more toxic than sulfanilamide. Marshall and Long³ have described the acute toxic effects following the intravenous injection of sodium sulfapyridine in rabbits and dogs. Their results have been entirely confirmed in experiments conducted in our laboratory.

The well-known serious complications which may follow sulfapyridine treatment, as hemolytic anemia, agranulocytosis, jaundice, etc., call for particular care in the use of this drug, but fortunately are rare. Vertigo, nausea, and vomiting are less dangerous, but are still very annoying side actions of the sulfapyridine treatment and occur much more frequently. In the following, an attempt was made to approach experimentally the possibility of alleviating these symptoms.

Experiments in Rabbits.—In order to secure clear-cut results with respect to toxic effects and their possible management, we have used in all experiments the intravenous injection of a 20 per cent sodium sulfapyridine solution. Such a solution has a pH of 10 or 11 and a high titrable alkalinity. One and one-half to two minutes were always consumed in administering the total dose. Male and female rabbits, weighing approximately 3 to 5 pounds, were used. While 500 mg. per kilogram regularly produced convulsions, 800 mg. per kilogram were fatal in 70 per cent of the animals. A total of 1,000 mg. per kilogram always caused death from secondary depression or pulmonary edema after convulsions. A total of 1,200 mg. per kilogram killed the animals within a few minutes following violent tonic or clonic convulsions. Thus, the picture of the acute poisoning presented itself as an initial marked stimulation, quickly followed by depression of the central nervous system. The convulsions were of the spontaneous type, and could not be provoked as a reflex to stimulation; in this respect they resembled picrotoxin convulsions. This observation suggested that they might be combated by means of hypnotics.

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Urethane was used in the first experiments. The minimum hypnotic dose for rabbits is 500 mg. per kilogram of this drug intravenously. The M.L.D. lies between 1,700 and 1,800 mg. per kilogram. The urethane was injected intravenously as a 25 per cent solution mixed with 1,000 mg. per kilogram of sulfapyridine. This amount of sulfapyridine alone would kill 100 per cent of the animals. The results obtained with varying amounts of urethane combined with this dose of sulfapyridine can be seen in Fig. 1 in the form of a dose mortality curve representing data on 35 animals. The mortality is zero with the use of 150 mg. per kilogram of urethane, an amount which, by itself, has no noticeable influence on a normal rabbit. In combination with sulfapyridine

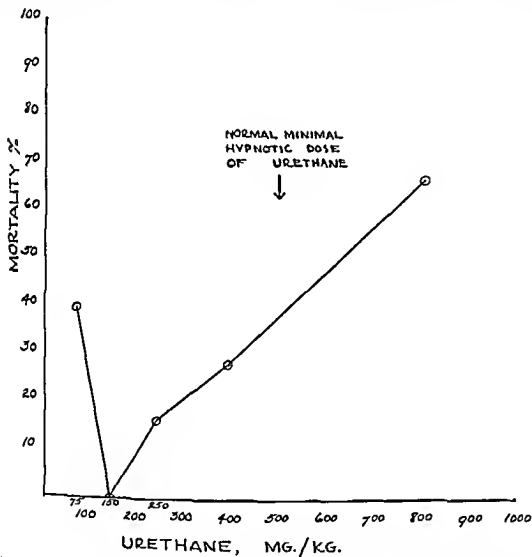


Fig. 1—Intravenous injection of sulfapyridine (1,000 mg. per kilogram) and urethane in rabbits.

there was slight twitching or occasional convulsions, but all six animals on this dose survived. This represents the optimal antidotal effect, since the mortality increases with either a larger or smaller urethane dose. With smaller doses, the protective action against the convulsions is insufficient; with larger doses, the convulsions disappear but a general depression quickly develops which leads eventually to respiratory paralysis. This death from depression occurs with doses of urethane which are innocuous to the normal animal, and seem to represent a summation of the depressant urethane effect and the second, depressant phase of sulfapyridine action.

Attempts to antidote 1,200 mg. per kilogram of sulfapyridine with a single urethane injection were unsuccessful. Repeated urethane injections, when made during the convulsive state of the sulfapyridine poisoning, occasionally saved the life of the animals.

It is well known that barbiturates are particularly effective in suppressing convulsions. The antidotal effect of sodium pentobarbital (nembutal) on sulfapyridine convulsions was, therefore, studied in a similar way. In rabbits 15 mg. per kilogram intravenously is the minimum hypnotic dose for nembutal and 45 mg. per kilogram the L.D. 50. Mixtures of different amounts of this drug with either 1,000 or 1,200 mg. per kilogram of sulfapyridine were injected intravenously. The results on 48 animals are given in Fig. 2. In general, the effect is similar to that of urethane, but a definite antidotal action is present even against the extremely high dose of 1,200 mg. per kilogram of sulfa-

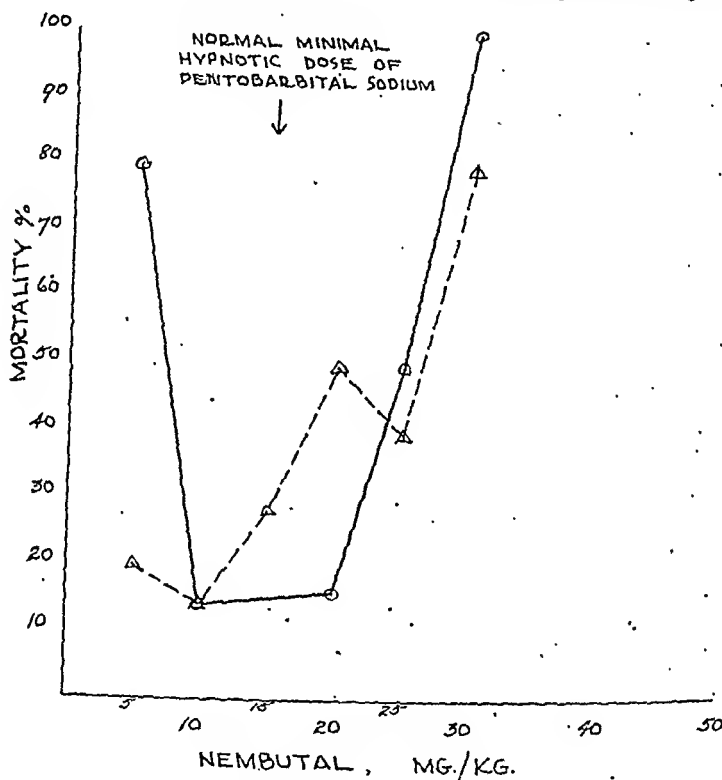


Fig. 2.—Intravenous injection of sulfapyridine and pentobarbital sodium in rabbits.

— 1,200 mg. per kilogram of sulfapyridine.
 - - - 1,000 mg. per kilogram of sulfapyridine.

pyridine. These curves also show an optimum antidotal effect at the normal hypnotic dose level of 10 mg. per kilogram. Smaller doses are unable to prevent convulsions, and larger ones, which are tolerated by normal animals, lead to respiratory paralysis. Finally, we conducted experiments using only 700 mg. per kilogram of sulfapyridine in order to see how high the dose of nembutal could be raised without producing cumulative depression. The maximum dose of nembutal which could be tolerated under these circumstances was 40 mg. per kilogram; therefore, with moderately high doses of sulfapyridine the toxicity of nembutal is not significantly enhanced.

The effect of large doses (1 to 3 Gm. per kilogram) of sodium bromide given orally on the convulsive action of 1,000 or 1,200 mg. per kilogram of sulfapyridine injected one-half hour later was also studied. Death could be pre-

vented only rarely, though the fatal outcome was somewhat delayed. Intravenous injection of magnesium sulfate (25 to 150 mg. per kilogram) or premedication with 35 mg. per kilogram of morphine subcutaneously were ineffective.

Experiments on Dogs.—While rabbits lend themselves only to the study of the more violent reactions, dogs show symptoms of sulfapyridine intoxication more like those seen in man. After the injection of 400 mg. per kilogram, unsteadiness of gait, signs of subjective discomfort, and repeated vomiting, frequently accompanied by urination and defecation were the rule. Twitching and, occasionally, convulsions developed. Convulsions were always present with 500 mg. per kilogram. Of 9 animals 2 died following this dose. Attempts to prevent the vomiting by the injection of 3 to 5 mg. of atropine sulfate fifteen minutes before the sulfapyridine proved to be entirely ineffective. Nembutal was added to the sodium sulfapyridine in varying amounts. The results are summarized in Table I. Ordinarily 15 mg. per kilogram is the minimum hypnotic dose, while 50 mg. per kilogram is the M.L.D. 50 of this barbiturate.

TABLE I

SODIUM SULFAPYRIDINE AND SODIUM PENTOBARBITAL (NEMBUTAL) INTRAVENOUSLY IN DOGS

CURRENT NO.	SODIUM SULFAPYRIDINE MG./KG.	SODIUM PENTOBARBITAL (NEMBUTAL) MG./KG.	NO. OF DOGS	NO. SURVIVED	REMARKS
1	400	-	2	2	Unsteadiness of gait, vomiting, defecating, convulsions
2	400	5	2	2	Atactic, vomiting, no convulsions
3	400	10	3	3	Slightly atactic, then relaxed. Two slept
4	400	15	1	1	Atactic, first restless, later quiet
5	400	30	2	2	Sleep
6	400	35	2	2	Sleep
7	400	40	4	2	One died of pulmonary edema, one of respiratory paralysis
8	400	50	3	2	One died of respiratory paralysis
9	500	-	9	7	Symptoms as under (1) but more severe
10	500	10	1	1	Atactic, vomiting
11	500	20	1	1	Light sleep

In Table I, 5 mg. per kilogram prevent convulsions but not vomiting, while 10 to 15 mg. per kilogram suppress not only the convulsions but also vomiting and the other signs of subjective discomfort. The dogs quieted down or even went into a light sleep. The sensitivity to nembutal appears to be slightly increased, since a dose of 40 mg. per kilogram of nembutal which is usually well tolerated may cause deaths in conjunction with sulfapyridine. The increase of the sulfapyridine dose to 500 mg. per kilogram necessitates a somewhat larger dose of nembutal to prevent toxic symptoms. Dogs in which convulsions had developed after the administration of sulfapyridine could be caused to sleep by the injection of a small amount of the barbiturate. These animals recovered promptly.

DISCUSSION OF RESULTS

The mortality curves of sulfapyridine in combination with either urethane or nembutal in rabbits seem to support the assumption that sulfapyridine has a

biphasic action upon the central nervous system. If a hypnotic is given in a dose high enough to prevent convulsions, the animal can survive this first stage of the sulfapyridine effect. It seems, however, that the convulsive and the depressant actions overlap to some extent, since a relatively small increase in the dose of the hypnotic rapidly brings about a summation of the depressant component. It is of particular interest to note that with nembutal, and especially urethane, subhypnotic doses are the safest and most effective.

If it is true that secondary death in depression is due to a combined action of the barbiturate and sulfapyridine, it should be possible to prevent these by the use of an analeptic. We have tried to prove the correctness of this assumption experimentally. It is known that the intravenous injection of picrotoxin is followed by its typical stimulating effect only after a latent period of a few minutes. Making use of this property, we have injected rabbits intravenously with 1,200 mg. per kilogram of sulfapyridine and added doses of nembutal sufficiently large to cause deaths when so combined with sulfapyridine. Picrotoxin in doses of 1 to 1.5 mg. was added to this mixture. None of four animals receiving a mixture of 1,200 mg. per kilogram of sulfapyridine, 30 mg. per kilogram of nembutal and 1.5 mg. per kilogram of picrotoxin died. But, as can be seen from Fig. 2, all animals died if no picrotoxin is added to such a mixture. Similarly, 2 of 3 animals survived when the sulfapyridine was mixed with 25 mg. per kilogram of nembutal and 1 mg. per kilogram of picrotoxin. None of these animals showed convulsions and all recovered promptly from what appeared to be a pure nembutal sleep. This experiment seems to give further support to the theory of a biphasic action of sulfapyridine.

Adriani⁴ has recently published experiments in which he tested the toxicity of the different anesthetics on rats which were pretreated with very high doses of sulfanilamide for three days. He found that while volatile and gaseous anesthetics, as well as avertin, were tolerated as well as they are by normal animals, the barbituates became anesthetic in subanesthetic doses and lethal in anesthetic doses. We repeated these experiments in rabbits and obtained similar results, provided very high doses of sulfanilamide were used (1,500 mg. per kilogram daily for three days). However, with moderately large doses of sulfapyridine (700 mg. per kilogram) in rabbits only a very slight augmentation of nembutal toxicity occurred, and this was also true for dogs. Our experiments are, of course, only partly comparable with Adriani's, since we studied only the acute effects of single doses. In this connection, it is interesting to note that W. L. M. King⁵ has been unable to find undesirable depression from ordinary sedative doses of barbiturates in pneumonia patients receiving sulfapyridine or sulfanilamide. We believe that the experimental results described in this publication justify the trial of sedatives in small doses in combination with sulfapyridine to alleviate irritating side effects of this drug upon the central nervous system.

SUMMARY

1. Death from intravenous lethal doses of sodium sulfapyridine in rabbits and dogs occurs during or after violent tonic or clonic convulsions.
2. Small doses of hypnotics, such as urethane or nembutal, can inhibit the convulsions and save life.

3. Subhypnotic doses of the urethane or nembutal are most effective; larger doses may cause death by adding to the secondary depressant action of sulfapyridine. Picrotoxin can overcome the effect of this summation.

4. Restlessness, nausea, and vomiting following large doses of sodium sulfapyridine in dogs can be completely eliminated by subhypnotic doses of nembutal.

5. The possibility of applying these observations to the alleviation of irritating side actions of sulfapyridine in man is discussed.

REFERENCES

1. Wien, R.: Toxicity of 2-(p-Amidobenzenesulfonamide) Pyridine, *Quart. J. Pharm. & Pharmacol.* 11: 217, 1938.
2. Marshall, E. K., Jr., Bratton, A. C., and Litchfield, J. T., Jr.: The Toxicity and Absorption of 2-Sulfanilamidopyridine and Its Soluble Sodium Salt, *Science* 88: 597, 1938.
3. Marshall, E. K., and Long, P. H.: The Intravenous Use of Sodium Sulfapyridine, *J. A. M. A.* 112: 1671, 1939.
4. Adriani, J.: Effects of Anesthetic Drugs Upon Rnts Treated With Sulfanilamide, *J. Lab. & Clin. Med.* 24: 1066, 1939.
5. King, W. L. M.: *Proc. Staff Meet., Mayo Clin.* 15: 21, 1940.

ADDENDUM.—After this paper was completed, corresponding work was done with sodium sulfathiazole. It was found that the lethal dose for dogs by intravenous injection lies between 200 and 1,000 mg. per kilogram. Thus it is less toxic than sodium sulfapyridine. We noticed, also, the absence of convulsions with doses below 800 mg. per kilogram, while sulfapyridine produces them with 500 mg. per kilogram. However, vomiting was observed with doses as low as 50 mg. per kilogram and in this respect it is more toxic than sodium sulfapyridine in dogs. On the other hand, most clinical reports indicate a lower incidence of nausea and vomiting with sulfathiazole. Encouraged by our results with nembutal in combating vomiting in dogs following sulfapyridine injection, we used this barbiturate also in combination with sodium sulfathiazole following the same technique as described above. Our optimal results were obtained if 20 mg. per kilogram of nembutal were injected intravenously simultaneously with 400 mg. per kilogram of sodium sulfathiazole. Three animals were used and all slept. No vomiting occurred. With dose levels of 200 to 500 mg. per kilogram of sodium sulfathiazole and 10 to 20 mg. per kilogram of nembutal we succeeded in suppressing the vomiting in about one-half the cases and decreasing its frequency in the other half without undue depression of respiration. Thus, principally, nembutal appears to exert the same effect in combination with sodium sulfathiazole as reported above with sodium sulfapyridine.

RECURRENCE IN PNEUMONIA

A CASE REPORT

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RECOVERY from pneumococcic pneumonia is usually associated with the appearance of specific antibodies in the patient's blood stream. The immunity thus produced is not predictable either in duration or in amount, as adjudged by the conventional units; and despite this temporary immunity recurrent attacks are more frequent in this than in any other acute infectious disease. It has even been suggested that one attack of pneumonia predisposes another. The high historical incidence of previous attacks (20 per cent in our experience) tends to support this theory. However, if predisposition bears the same relation to type specificity as does immunity, a study of the types of pneumococci found in recurrent attacks of pneumonia lends the theory no support. In the four-year period 1935-1939, only four patients have been admitted to the Cincinnati General Hospital with recurrent attacks of pneumonia due to an homologous type of pneumococcus.¹ Of these pneumonias, two were due to type I pneumococcus, one to type II, and one to type VIII.

The influence of the carrier state on recurrences is not known but deserves further consideration. Finland and Winkler² collected a series of 57 cases from which they concluded that one attack of pneumonia did not confer permanently increased resistance against subsequent infections, even though of homologous types. (However, second infections rarely occurred within less than a year.) They also found that the duration and mode of termination, the distribution of the lesions and pneumococcic types were very similar in initial and recurrent pneumonias, and that bacteriemia occurred almost as frequently in second infections as in first. The later infections were found to differ only in two respects namely, that bilateral involvement was twice as frequent, and bronchopneumonia was encountered chiefly with the recurrences. These authors agreed that the acquisition of circulating type-specific antibodies and tissue immunity following one attack of pneumonia was only a temporary state and did not preclude recurrent pneumonia.

In experimental pneumonia of dogs, Coggeshall and Robertson³ have found that one infection confers increased resistance to a second, a resistance which lasts a varying number of months. This property is demonstrated by the fact that lethal dosages of organisms were used in producing a recurrent pneumonia at the site of the previous infection. With second infections, this injection was

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made five days after the termination of the disease. If delayed, however, until two weeks after recovery, the dog died. After five or six attacks of pneumonia, the injection could be delayed three or four months with impunity. Lethal dosages injected fifteen to twenty-five days after the last infection into a previously uninvolved lobe resulted in death. Even with the greater dosages of organisms, the recurrent infections were milder, and the febrile course was briefer (two to three days). Bacteriemia dropped from 54 per cent in primary pneumonias to 5.6 per cent in recurrences.

In attempting to define the exact mechanism of this increased resistance, Coggeshall and Robertson found the leucocytic response prompt and adequate in both primary and recurrent infections. Excess of circulating antipneumococcic immune bodies varied greatly, some dogs apparently having none, though they recovered from their disease with rapidity.

The only marked difference that these authors found in primary and recurrent pneumonias was in the microscopic appearance of the lesions. Recurrent lesions in previously involved areas were invaded early by large numbers of macrophages, such as were found ordinarily in areas of resolution, and the pneumococci were found in either greatly diminished numbers or not at all. Large concentrations of interalveolar macrophages were to be found for five days following recovery; then they gradually diminished until, on the fifteenth day, they were no longer noticeable. Recurrent infections in previously uninvolved areas resembled primary infections produced with nonfatal doses of organisms, the macrocytic response occurring later than in the above.

Briefly, then, Finland and Winkler from their cases found no evidence of increased local or type-specific resistance. Coggeshall and Robertson, on the other hand, found a characteristic transitory change in the fixed tissue cells consisting of early localization of septal and perivascular cells and the rapid disappearance of injected organisms. For the period of its stay, this change gave increased resistance against a second infection.

In view of the preceding facts, it is interesting to speculate on the relationship of the several illnesses related in the following case report of a patient with recurrent type VIII pneumonia associated with meningitis.

CASE REPORT

A. R., a thirty-six-year-old colored woman, entered the Cincinnati General Hospital July 25, 1934. A right mastoidectomy was performed as an emergency operation. The laboratory reported a nonhemolytic gram-positive coccus cultured from the swab taken at operation; further identification was not attempted.

Three years later the patient was admitted to the Surgical Ward of the same hospital, where a colostomy was done July 15, 1937, to relieve a rectal stricture secondary to lymphogranuloma inguinale. Postoperatively, the patient developed a pneumonia in the right lower and middle lobes, with a white blood cell count of 23,600. No attempt was made to learn the etiology; treatment was symptomatic. A month later (on August 23) x-ray examination showed a localized area of density, 7 by 9 cm., which was interpreted as fluid in the lower posterior portion of the right chest. On direct examination of the aspirated pus, pneumococcus VIII was identified. The sputum also showed pneumococcus VIII on direct examination; the blood culture at this time, however, was negative. X-ray examination four weeks

later (September 21) showed a small amount of fluid still present, there being a definite fluid level. Two attempts at thoracentesis were unsuccessful. An x-ray examination of the chest taken just before dismissal on October 19, two and a half months after the original pneumonia, showed only evidence of thickened pleura. The right dome of the diaphragm was flattened and was adherent to the right lateral chest wall.

On February 13, 1939, the patient went to bed with influenza-like symptoms. Her husband stated that she had fever at this time and during the next few weeks she had seven or eight chills, several of them occurring during the week previous to admission. Headache (from which she had suffered since the mastoidectomy) became more severe, and was frontal in character. There was no vomiting and no earache. One week before entry (March 11), she developed a cough productive of a thin white sputum. By March 16 the severity of the cough had lessened. Her husband noticed that her hand strayed to the right side of head and neck, but she denied earache, merely stating that she had difficulty moving her head. Thirty-six hours before entrance to the hospital she stopped talking; her neck became stiff at that time and her eyes turned to the left.

Physical Examination.—On admission March 18, 1939, examination showed a critically ill, comatose, middle-aged negress in the opisthotonos position. Respirations were rapid and labored. Pulse rate was 160, of poor quality, but regular. Head and eyes were directed to left. The right corneal reflex was diminished, the left was absent; pupils were fixed to light. There was no facial asymmetry. The ears showed no evidence of recent infection. The neck was rigid. The heart was apparently normal. Chest findings were not remarkable except for dullness in the right lower axilla. The colostomy in the left lower quadrant seemed to be working well.

The tone of the right arm was questionably greater than that of the left. Focal tremors occurred in the right arm and leg, occasionally spreading to the left. Reflexes were diminished in the arms, and were normal in the legs. Plantar responses were absent. Brudzinski's and Kernig's signs were present.

Accessory examinations showed a leucocytosis of 21,350, with a definite shift to the left. Lumbar tap yielded cloudy fluid under an initial pressure of 110 mm.; of the 15,000 white blood cells, 98 per cent were polymorphonuclear leucocytes. Pneumococci type VIII were seen on direct examination of the spinal fluid. A blood culture showed countless colonies of pneumococcus VIII per cubic centimeter. A culture taken from the throat also showed pneumococcus VIII in moderate numbers.

History Course.—During the succeeding thirty-four hours the patient was given 17.5 Gm. of sodium sulfapyridine intravenously and fluids subcutaneously without any noticeable change in the course of the illness. Despite levels of sulfapyridine of 17.7 mg. per cent in the blood and 14.8 mg. per cent in the cerebrospinal fluid, and a sterilization of the blood stream, she died.

Autopsy revealed acute endocarditis, involving the mitral and tricuspid valves; acute purulent leptomeningitis; old organized lobar pneumonia of the right upper lobe; elephantiasis of labia majora; moderate rectal stricture; double-barreled colostomy; evidence of old right mastoidectomy; old right obliterative pleuritis; and toxic changes of the viscera.

Pneumococci VIII were identified from the spinal fluid and from the right upper lobe lung tissue on direct examination. Blood culture taken twelve hours post mortem showed no growth. Blood broth culture of the tricuspid vegetation after two weeks' preservation in 10 per cent formalin yielded pneumococcus VIII. Only the tricuspid vegetation was cultured, the mitral vegetation being saved for histologic inspection.

COMMENT

Careful bacteriologic study was not made of the original organism isolated from the mastoid. However, since the description of the organism permits the consideration of a pneumococcus, it might be noted here that pneumococcus VIII is not infrequently found in the ear and paranasal sinuses.

Three years later the patient had a clinically atypical pneumonia due to pneumococcus VIII; this was complicated by empyema. Eighteen months later she was readmitted with a pneumococcus VIII infection of the lung, endocardium, and meninges. It is interesting to note that this second pulmonary infection did not involve the previously involved lobes, raising the speculation that there may have been a residual tissue immunity within the lung⁴ from the first infection, despite the apparent breakdown in general immunity. Unfortunately, no bacteriologic or immunologic studies were made during intervals of apparently good health. But in view of the fact that pneumococcus VIII was found in the patient's sputum and pleural fluid at a time when she had no clinical evidence of pulmonary disease, it seems a not unfair assumption that she remained a carrier after she had recovered from the acute pneumococcal pneumonia. The implication must be that she erected sufficient immunity defenses to recover from the pneumonia and to hold the organism in abeyance even though it was still present in the respiratory tract and pleural cavity.

Finland and Tilghman,⁵ studying pneumococcic infections in families and other closely-knit groups, found that healthy carriers of disease-producing pneumococci may develop type-specific antibodies. This raises the question whether the carrier state may be desirable in convalescent pneumonias; or whether the repeated or continuous antigenic effect of the saprophytic organisms depresses the formation of antibodies, a condition frequently seen in hyperimmunized horses. This supposition scarcely seems plausible in view of the infrequency of homologous recurrences and the relatively high incidence of carriers among convalescents for varying periods following the acute infection.

Despite our incomplete knowledge concerning the pathogenesis of pneumococcal pneumonia and immunity to it, the carrier state appears to constitute a potential danger not only to others, but also to carriers, and as such should be a point of attack in pneumonia programs.

SUMMARY

A case of recurrent pneumonia due to the homologous type of pneumococcus is reported, with the implication that it probably represents a self-infection.

REFERENCES

1. Hamburger, M., and Ruegsegger, J. M.: Recurrence of Type I Pneumonia After Sulfapyridine Therapy, *New England J. Med.* 221: 445, 1939.
2. Finland, M., and Winkler, A. W.: Recurrence in Pneumococcus Pneumonia, *Am. J. M. Sc.* 188: 309, 1934.
3. Coggeshall, T. T., and Robertson, O. H.: A Study of the Repeated Attacks of Experimental Pneumococcus Lobar Pneumonia in Dogs, *J. Exper. Med.* 61: 218, 1935.
4. Robertson, O. H.: Recent Studies on Experimental Lobar Pneumonia, *J. A. M. A.* 111: 1432, 1938.
5. Finland, M., and Tilghman, R. C.: Bacteriological and Immunological Studies in Families With Pneumococcic Infections, *J. Clin. Investigation* 15: 501, 1936.

FURTHER OBSERVATIONS ON THROMBOCYTOPEN*

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FROM the spleens of three successive persons with idiopathic thrombocytopenic purpura a substance was extracted which reduced the number of platelets in the circulating blood of rabbits. This substance was called thrombocytopen, and was described in detail in 1938.¹ In the subsequent ten months, only one other patient suffering with the disease has come to operation in the hospital. The extract from the spleen of this patient not only corroborated and substantiated the observations made with the former preparations, but it also opened up new paths of investigation. In addition, four more control spleens have also been extracted and tested.

MATERIAL AND METHOD

CASE.—A. W., a white married woman, aged 24 years, was admitted to the Johns Hopkins Hospital on September 17, 1938, complaining of excessive bleeding. Her family history revealed a maternal aunt who had "purpura," but was successfully treated with snake venom. Her past history is of little interest. Her menstrual periods were always regular, occurring every twenty-six days, and lasting seven days, with variable flow. About five years before admission she began to notice hemorrhages around her gums and became aware of the fact that she bruised easily. A year and a half later she was delivered spontaneously of a full-term normal child. The exact amount of blood loss at that time is unknown, but the patient was transfused with 500 c.c. of blood and remained well for six months, after which time the bleeding recurred. Three years ago moccasin venom was started (dosage unknown) and continued for six months, with some alleviation of symptoms. Two months after cessation of treatment the hemorrhages recurred. One year ago venom therapy was begun once more and was continued for two months without benefiting the condition. In view of the unfavorable response, the patient was given a 500 c.c. blood transfusion. In December, 1937, deep x-ray therapy over the spleen was instituted, 1,750 r. in air being given anteriorly, and 750 r. posteriorly, over the splenic region. Following this treatment she was considerably improved until July, 1938, when weakness returned and petechial hemorrhages once more appeared. In the two months prior to her admission she was given three 500 c.c. blood transfusions as well as liver extract. Since the onset of her illness she has had many fainting attacks which were usually followed by an exacerbation of her purpura. The platelet count on August 4, 1938, was 170,000, and on August 30, it was 180,000. Physical examination of the patient was essentially negative. There were no evidences of hemorrhage in the skin or gums. The spleen was not palpable. Blood studies made on September 19 revealed leucocytes 5,050, hemoglobin 93 per cent, red blood cells 4.73 million, and platelets 80,000. The clotting time was normal, the bleeding time was eleven minutes. The clot was nonretractile. The tourniquet test was negative, as was also the routine Wassermann on the blood serum. On September 20, splenectomy was performed by Dr. Warfield M. Firor. The patient recovered from the operative procedure satisfactorily. There was no excess of bleeding either at operation or afterward. The platelet count three hours after operation was 166,000. During the period of her stay in the hospital it remained around 150,000. The bleeding time was less than four and one-half minutes. The patient was discharged on October 3, symptomatically well.

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The spleen weighed 95 Gm. and looked grossly normal. Microscopically there were many large Malpighian corpuscles with germinal centers. Final pathologic diagnosis: spleen showing nonspecific changes (thrombocytopenic purpura).

The preparation of the spleen began on the day of operation, when the organ was ground up and placed in twice its volume of reagent acetone. One month later, on October 25, one-half of the extract was removed and filtered, and evaporation of the acetone was carried out by reduced pressure under a Bell jar. This step in the preparation of the extract differed from the previously published method in which distillation of the acetone was carried out under reduced pressure with the application of some heat. It was thought that even a small amount of heat might affect the potency of the preparation, and in fact a more potent product did result when heating was omitted.

When evaporation was complete, the usual dark brown, gummy residue was left. This was suspended in 100 c.c. of distilled water. The dilution of this extract was, therefore, twice that obtained from previous spleens, since formerly the same amount of distilled water was added to the acetone-extracted material from an entire spleen.

The extract was injected in amounts varying from 0.5 to 10 c.c. into 10 rabbits, all of which died in periods ranging from one hour to twenty-four hours after the injection. In the animals which survived more than four hours after injection, it was noted that the platelet count fell significantly. The direct method of counting platelets with the fluid of Rees and Ecker was used, and every count was controlled by the indirect blood smear procedure.

The remainder of the extract was now run through a Seitz filter and further injections were made. Subsequently all extracts were Seitz filtered before injection.

RESULTS

Experiment 1. A male rabbit (R-13), weighing 2.4 kg., was injected intravenously with 1 c.c. of the extract from patient A. W. on December 28, 1938. The blood platelets fell from 610,000 to 283,000 in six hours, and hovered in the neighborhood of 280,000 during the subsequent eighteen hours. Another 1 c.c. of the extract was injected on December 29, the platelets varying from 213,000 to 280,000 during the next twenty-four hours. On December 30 the platelets began to fall to lower levels without further injection, and reached the low count of 94,000 approximately fifty-four hours after the first injection. By the morning of the following day the platelets had risen precipitously, reaching a high count of 6,029,000. Many huge, abnormal appearing platelets were seen. Smears showed enormous numbers of platelets at this time. During the period of study the red blood cells did not vary significantly from the normal. Leucocytosis was practically constant, the white blood cell count being in the neighborhood of 20,000, with an excess of adult polymorphonuclear forms. On January 1 the animal died. Autopsy revealed no hemorrhages. There was patchy consolidation of both lungs. Sections of the femur bone marrow revealed hyperplasia of the myeloid elements. No abnormalities could be found in the megakaryocytes.

Experiment 2. On January 25, 1939, 1 c.c. of the same extract was injected into another male rabbit (R-17), weighing 2.2 kg. A control specimen of bone marrow had been removed from this animal by biopsy nineteen days previously. Table I shows behavior of platelets after injection. The white blood cells had risen from an original count of 4,950 to nearly 15,000 on the second day, and gradually fell to a normal level three days later. The red blood cell count did not vary significantly from an original count of 4.78 million. On February 2, 1939, the animal died. Autopsy revealed patchy consolidation of lungs. Sections of the spleen revealed small foci of myeloid tissue with disproportionately great numbers of megakaryocytes, and these cells appeared in the liver as well (Fig. 1). There was no evidence of platelet formation in the lungs. The femur bone marrow showed no detectable abnormalities either in the number or in the morphology of megakaryocytes.

Experiment 3. On February 23, 1939, a male rabbit (R-15), weighing 1.8 kg., was injected with 1 c.c. of the extract. A bone marrow biopsy had been made six weeks previously. The platelets fell characteristically (Table I) from the preinjection level of 628,000 to a low of 55,000 three days later. Eight days after the original injection the platelets had risen

only to 217,000, and the animal was sacrificed. No noteworthy changes were found in the megakaryocytes of the femur marrow, but the spleen again showed many megakaryocytes, the liver a few. The lungs and other organs were normal.

Meanwhile, on February 1, 1939, the second half of the acetone extract from the spleen of A. W. was prepared for use by evaporation of acetone and suspension of the residue in distilled water. This was not used, however, until March 20, 1939, after an interval of seven weeks. It had, however, been kept in the refrigerator at a temperature of about 40° F.

TABLE I

Rabbits R-15 and R-17 received intravenously 1.0 c.c. of the splenic extract, while rabbit R-29 was given 2.0 c.c. Rabbit R-26 served as a control.

HOURS AFTER INJECTION	PLATELETS $\times 10^5$			
	R-17	R-15	R-26	R-29
0	6.52	6.28	6.23	5.71
1	5.09	5.59	-	-
2	4.08	2.42	-	-
5	3.17	2.01	5.64	-
7	2.72	-	4.75	2.34
24	1.57	1.18	5.24	0.70
32	1.02	1.02	6.08	2.05
48	0.89	0.88	5.63	1.27
55	0.74	-	5.31	1.35
72	0.49	0.55	5.60	1.00
75	2.35	-	4.90	-
96	0.76	1.10	5.41	3.21
120	0.93	1.02	5.93	0.72
144	1.06	1.81	6.81	6.25
168	-	2.17	5.58	-
192	-	-	5.81	-
216	-	-	6.92	-

Experiment 4. On March 20, a male rabbit (R-19), weighing 2.1 kg., was given two injections of 0.5 c.c. each of this second preparation of extract from A. W., without a significant change from the preinjection level of 620,000 platelets. The following day another 1.0 c.c. was given, and the platelets reached a low of 453,000 three hours after the injection. For the next three days the platelets varied between 252,000 and 550,000, when another 1 c.c. of extract was injected. They fell again to 232,000 and remained at 200,000 to 414,000 until March 27, when the platelet count reached a high of 729,000. Two cubic centimeters of the extract were then injected, and the platelets rose to 1,624,000 and then to 3,629,000 two and six hours, respectively, after injection. Mostly large, abnormal platelets were seen. By the next morning the platelets had fallen precipitously to 332,000, then rose rapidly and steadily to 7,805,000 at 4:00 P.M. and to 9,450,000 at 11:30 P.M. Smears made at this time showed great masses of platelets such as those illustrated by Bunting.² The white blood cell count at this time was 20,000 and the red blood cell count was 5.02 million. There was nothing unusual about the smear except for the platelets. On the following day (March 29) the platelets, which were 848,000 in the morning, had reached 3,065,000 sixteen hours later. A maximum leucocyte count of 32,150 was found at this time.

For the next two days the platelets varied between 204,000 and 821,000, and the animal was killed. Post-mortem histologic sections of organs revealed no detectable abnormalities. Differential counts were attempted on megakaryocytes of femur marrow, but no significant change was noted when compared with control sections.

Experiment 5. Another male rabbit (R-27), weighing 2.4 kg., was treated exactly as was rabbit R-19 and reacted in the same way. Control biopsies of spleen and bone marrow had been taken three weeks before injections were begun, but no anatomic abnormalities could be made out in the post-mortem sections.

Experiment 6. To eliminate the possibility that the many small bleedings necessary for counting platelets were responsible for the changes in the blood picture of all the animals or

for the presence of megakaryocytes in the spleen and liver of two of the earlier animals, a control rabbit R-26 was bled in amounts varying from 0.2 to 1.0 c.c. three times daily for ten days (Table I). The platelets varied from 692,000 to 475,000, while the other formed elements of the blood did not vary significantly. The animal was sacrificed on the tenth day. Histologic sections of femur marrow and spleen made post mortem did not differ from biopsy specimens taken from the same animal three weeks before the bleedings were begun.

Animals used for all subsequent experiments underwent biopsies of spleen and femur bone marrow at least three weeks before any injections were made.

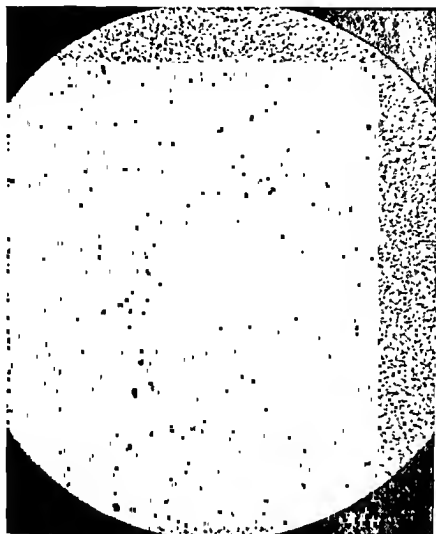


Fig 1—Section of spleen from rabbit R-17 showing a large number of megakaryocytes which normally are not present in the spleen (H. & E. $\times 7$).

Experiment 7. On April 3, 1939, a male rabbit (R-29), weighing 1.7 kg., was injected with 2.0 c.c. of the second half of the extract (Table I). The platelets fell from a control level of 571,000 to 78,000 in fifteen hours, and for the next five days varied from 70,000 to 321,000 without further injection. Then they slowly began to rise until on April 9, six days after the injection, the count had risen to a normal of 625,000, and the animal was killed. Sections of the spleen showed occasional megakaryocytes, but no abnormalities could be detected in other organs.

Experiment 8. Another male rabbit (R-30), weighing 1.8 kg., was injected with 2.0 c.c. of extract on April 11, 1939. The platelets fell from a normal of 545,000 to a low of 161,000 on the day of injection. On the following day they rose from 1.06 million to 2.86 million. This same day, eight hours after the count of 2.8 million, the platelets had fallen to 172,000. On the following day (April 13) they rose from 133,000 to 576,000, and the animal was killed. No significant post-mortem histologic findings were observed.

Experiment 9. A female rabbit (R-31), weighing 3.3 kg., was now used. It seemed evident that the extract was losing potency, so 2 c.c. were injected on April 25 without effect, and another 2 c.c. were given sixteen hours later. The platelets then fell to 107,000. They were around 200,000 for two days, when 2.0 c.c. were again injected without further

effect. Subsequently 2.0 c.c. and then 4.0 c.c. were injected separately in the next four days, but the platelets continued to remain around 200,000. The animal was then sacrificed. Sections revealed no abnormalities.

Experiment 10. In those animals studied during May, 1939, as much as 16 c.c. of the extract given over a two-day period in doses of 8 c.c. succeeded in bringing platelets down only to 191,000. It became even more evident that the extract was losing strength.

Experiment 11. On May 29 a male rabbit (R-16), weighing 2.6 kg., was injected with 8 c.c. of the extract. Platelets fell only to 421,000 fifteen hours after injection. The last 20 c.c. of the extract were then injected. The following day the platelets were counted at 419,000 and slowly rose to 592,000. The animal survived and subsequent counts were normal.

CONTROL EXPERIMENTS

Spleens removed at operation from four patients suffering with conditions other than thrombocytopenic purpura were extracted and studied. Three of these were spleens removed for Banti's syndrome, while one was a normal spleen removed because of rupture as a result of trauma. The extracts were prepared in the manner described, but on injection into rabbits of amounts up to 40 c.c. no significant alterations in the blood platelet level were produced.

COMMENT AND DISCUSSION

The preparation of thrombocytopen, as described in this report, presents two significant differences from that previously mentioned.¹ In the first place, removal of the acetone from the extract was accomplished by distillation under reduced pressure with the application of a small amount of heat. This method, however, presented two difficulties. First, as more and more acetone was distilled off, the residue became thicker and thicker, and it was only by great effort and careful regulation of applied heat that the material was prevented from bubbling up through the distillation system. There was no doubt that some of the extracted material remained in the system. Second, it was not known whether or not thrombocytopen was heat stable, and hence some of its potency in the early extracts might have been lost by heating. With the new method of removal of acetone by evaporation under reduced pressure in a Bell jar, it was possible to obtain a residue rich in thrombocytopen to which no heat had been applied.

The second difference in preparation was in use of the Seitz filter. Although ordinary sterile precautions were taken, and although the extract was always kept in the refrigerator, bacterial and other contamination necessarily occurred sooner or later. It was as an added precaution, then, that Seitz filtration was carried out before injections. This procedure ruled out the possible effect of specific³ as well as nonspecific organisms.

The action of the present extract differed somewhat from that previously reported. For the most part, the production of thrombopenia followed the pattern established in the early experiments of Troland and Lee, but it was more prolonged, lasting in the case of rabbit R-17 seven days after the injection of 1 c.c. Furthermore, the effect of the extract was variable in that in some cases a secondary thrombocytosis was encountered. Whether this reaction was compensatory to the primary diminution in the platelet level, or whether it indicated some stimulatory effect of the extract on the thrombopoietic system, could

not be ascertained. Elevation of the blood platelet count above the normal has been observed experimentally under many different conditions. Bedson⁴ noted prolonged thrombocytosis following splenectomy in rabbits. In the same species thrombocytosis has been observed following injections of bacillus thrombo-cytogenes,³ following experimental productions of subcutaneous abscesses,⁵ and after the administration of certain drugs such as pyrodine,^{6, 7} trypan blue,⁷ phenylhydrazine,⁸ turpentine and saponin.² Inhalations of carbon monoxide and carbon dioxide by rabbits also resulted in temporary increase in the number of platelets.^{9, 10} The effect of ultraviolet rays in raising the platelet count has been demonstrated by Steiner and Gunn¹¹ and by Toyoda.¹² Bedson⁴ and Koster¹³ described thrombocytosis after "blockade" of the reticulo-endothelial system with India ink. Clinically, thrombocytosis has been observed frequently following splenectomy, and platelet counts as high as 5 or 6 million have been reported.^{14, 15}

In none of the reports in the literature has thrombocytosis approached a platelet level of 9,450,000, as observed in rabbit R-19. In this rabbit, and in the other animals displaying unusually high platelet counts, abnormally large and irregularly sized platelets were frequently seen in the counting chamber and in smears. This phenomenon has been previously described by Bedson¹⁶ and others.

No light has as yet been thrown on the precise manner in which thrombo-cytopen exerts its influence on the experimental animal. In two of the earlier animals, R-17 and R-15, in which the platelet counts were maintained at low levels for seven and eight days, respectively, post-mortem sections of the spleen showed megakaryocytes in great abundance. That they may occur normally in small numbers in adult rabbits is pointed out by Pianese.¹⁷ Definitely increased numbers were noticed by Bunting,¹⁸ and by Firket and Campos,¹⁹ following intravenous injection of saponin in rabbits. Pugliese²⁰ noted the same phenomenon in hedgehogs following repeated bleedings. To rule out the possibility that the presence of megakaryocytes in the spleens of rabbits R-17 and R-15 was due to the repeated small bleedings necessary for blood studies, another animal, rabbit R-28, was subjected to venepuncture and removal of blood in like quantities for a period of ten days. This animal received no thrombocytopen, and sections made from the spleen and bone marrow removed at autopsy could not be distinguished from control sections obtained from biopsy specimens one month before the experiment began. Platelets in this animal were counted at least three times a day during the ten-day observation period. They were found to vary from 475,000 to 692,000.

The prolonged effect of thrombocytopen in some of the experiments being reported suggested the possibility that the extract might affect the megakaryocytes directly. The condition of the bone marrow and megakaryocytes in idiopathic thrombocytopenic purpura has never been adequately settled. Jedlička and Altshuller,²¹ and Papp and Antognini²² noted morphologic changes in the megakaryocytes of bone marrow in several cases of purpura. Willis²³ studied megakaryocytes during different phases of thrombocytopenic purpura. He noted no variations in megakaryocytes, and saw no sign of platelet formation in severe forms of the disease. In 6 cases of thrombocytopenic

purpura studied by Lawrence and Knutti²⁴ megakaryocytes were normal in number and diminished in 2. Vogel, Erf, and Rosenthal²⁵ observed no variations in quantity or quality of megakaryocytes. In our own studies no numerical or morphologic changes could be detected in the bone marrow giant cells, either in animals whose platelets had suffered a reduction in number, or in those whose platelets had reached very high levels. Observations made by supravital technique or on fixed material stained by Wright's or hematoxylin and eosin failed to reveal significant alterations from the bone marrow picture shown in biopsy material taken before experimental injections of thrombocytopen were made. The mode of action of the extract is, therefore, still unsettled.

It appears from the experiments that the potency of the extract, as preserved in aqueous solution, diminishes with time. The earlier experiments from December to early April (three to seven months after splenectomy) showed an activity of the extract which was fairly uniform in that a given dose produced a diminution in platelets which was sometimes followed by a thrombocytosis. Later, however, gradually increasing doses were required, and finally even large doses had no effect. Whether this apparent decrease in potency could have been prevented by keeping the extract either in acetone or in the dry state was not determined.

In recent months a number of investigators²⁶⁻³⁰ have published reports dealing with failure to isolate thrombocytopen from spleens of patients with thrombocytopenic purpura. It is impossible to explain this apparent disparity in results. A point of interest arises, however, in the fact that many spleens extracted by the afore-mentioned workers were significantly heavier than those of Troland and Lee¹ or the one in the present report. The question arises whether there may not be several forms of idiopathic thrombocytopenic purpura depending on the size of the spleen and the presence or absence of an extractable platelet-reducing substance. Even clinically, a large spleen has raised doubts regarding the correct diagnosis of thrombocytopenic purpura according to Wiseman.³¹ Hobson and Witts³² recently found some platelet-reducing activity in the extract of a spleen from a case of purpura hemorrhagica.

SUMMARY

A preparation of thrombocytopen from a patient with idiopathic thrombocytopenic purpura caused reduction of blood platelets in rabbits. In some cases a marked thrombocytosis followed, and in several experiments large numbers of megakaryocytes could be found in the spleens of the animals studied.

Four more control spleens, extracted in a similar manner, proved to have no effect on blood platelets.

The potency of an aqueous solution of thrombocytopen diminishes with time.

REFERENCES

1. Troland, C. E., and Lee, F. C.: *Thrombocytopen*, J. A. M. A. 111: 221, 1938.
2. Bunting, C. H.: *Blood Platelets and Megakaryocytes in Hodgkin's Disease*, Bull. Johns Hopkins Hosp. 22: 114, 1911.
3. Gunn, F. D.: *Reactions of the Bone Marrow in Experimentally Induced Thrombocytosis*, Arch. Path. 12: 153, 1931.

4. Bedson, S. P.: The Role of the Reticulo-Endothelial System in the Regulation of the Number of Platelets in the Circulation, *Brit. J. Exper. Path.* 7: 317, 1926.
5. Gucci, G.: Il comportamento delle piastrine in alcune malattie chirurgiche, *Policlinico (sez. chir.)* 40: 141, 1933.
6. Poletti, B.: Sur la préexistence des plaquettes dans le sang circulant, *Arch. ital. de biol.* 73: 39, 1924.
7. Mariconda, G.: Osservazioni intorno alla origine istiocitaria delle piastrine del sangue, *Arch. di fisiol.* 32: 387, 1933.
8. Rosenthal, F., Wislicki, L., and Kollek, L.: Hydroxylaminderivat als Blutgifte und Eiweissabbauprodukte, *Klin. Wchnschr.* 7: 1514, 1928.
9. Bianchini, G., and Fabbri, S. M.: Le piastrine nel sangue degli animali avvelenati con co e veleni ematici, *Pathologica* 14: 230, 1922.
10. Bianchini, G., and Pagni, G.: Le piastrine nel sangue degli animali avvelenati con CO₂, *Pathologica* 14: 235, 1922.
11. Steiner, P. E., and Gunn, F. D.: The Response of Blood Platelets to External Stimuli: Ultraviolet Light, Iodine and Coal Tar, *Arch. Path.* 11: 241, 1931.
12. Toyoda, G.: Splenic Hormone and Its Influence on Thrombocytopoiesis After Ultraviolet Ray Radiation, *J. Chosen M. A.* 22: 45, 1932.
13. Koster, H.: The Relation of the Reticulo-Endothelial System to the Blood Platelet Count, *J. Exper. Med.* 44: 75, 1926.
14. Evans, W. H.: Blood Changes After Splenectomy in Splenic Anaemia, Purpura Haemorrhagica and Acholic Jaundice, With Special Reference to Platelets and Coagulation, *J. Path. & Bact.* 31: 815, 1928.
15. Tocantins, L. M.: The Mammalian Blood Platelet in Health and Disease, *Medicine* 17: 153, 1938.
16. Bedson, S. P.: An Enquiry Into the Genesis of the Mammalian Blood Platelet, *J. Path. & Bact.* 26: 145, 1923; 28: 114, 1925.
17. Fanese, G.: Per una miglior conoscenza dei megacariociti, *Haematologica* 1: 61, 1920.
18. Bunting, C. H.: Experimental Anemias in the Rabbit, *J. Exper. Med.* 8: 625, 1906.
19. Firkin, J., and Campos, E. S.: Generalized Megalocaryocytic Reaction to Saponin Poisoning, *Bull. Johns Hopkins Hosp.* 33: 271, 1922.
20. Pugliese, J.: Ueber die physiologische Rolle der Riesenzellen, *Fortschr. d. Med.* 15: 729, 1897.
21. Jedlička, V., and Altschuller, G.: Chronic Essential Thrombopenia (Frank); Report of 2 Cases With Remarks About Its Pathogenesis, *Acta. med. Scandinav.* 64: 123, 1926.
22. Papp, C., and Antognini, G.: Contributo allo studio delle porpore trombopeniche, *Arch. Ist. biochim. ital.* 4: 259, 1932.
23. Willi, H.: Über den Bau und die Funktion der Megakaryocyten und ihre Beziehungen zur thrombopenischen Purpura, *Folia haemat.* 53: 426, 1935.
24. Lawrence, J. S., and Knutti, H. E.: Bone Marrow in Idiopathic Thrombopenic Purpura, *Am. J. M. Sc.* 188: 37, 1934.
25. Vogel, P., Erf, L. A., and Rosenthal, N.: Hematological Observations on Bone Marrow Obtained by Sternal Puncture, *Am. J. Clin. Path.* 7: 436, 1937.
26. Pohle, F. J., and Meyer, O. O.: Inability to Demonstrate a Platelet Reducing Substance in an Acetone Extract of the Spleen From Patients With Idiopathic Thrombocytopenic Purpura, *J. Clin. Investigation* 18: 537, 1939.
27. Major, R. H., and Weber, C. J.: Is There a Platelet-Reducing Substance in the Spleen of Thrombocytopenic Purpura? *J. LAB. & CLIN. MED.* 25: 10, 1939.
28. Tocantins, L. M.: No Platelet-Destroying Action in Extracts of the Spleen and Urine of Patients With Chronic Thrombopenic Purpura, *Proc. Soc. Exper. Biol. & Med.* 42: 485, 1939.
29. Hodge, I. G., and Strong, P. T.: The Effect of Splenic Extracts of Patients With Thrombocytopenic Purpura on the Platelet Count of Rabbits, *Bull. Ayer Clin. Lab., Pennsylvania Hosp.* 3: 267, 1939.
30. Doan, C. A.: A Summary of Investigative Work in the Department of Medicine, 1938-39, *Proc. Sixth Post-Collegiate Clin. Assembly, College of Medicine, Ohio State University*, p. 43, 1939.
31. Wiseman, B. K.: The Relationship of the Cytolytic Functions of the Spleen to the Blood Dyscrasias, *Proc. Sixth Post-Collegiate Clin. Assembly, College of Medicine, Ohio State University*, p. 174, 1939.
32. Robson, F. C. G., and Witts, L. J.: Platelet-Reducing Extracts of the Spleen, *Brit. M. J.* 1: 50, 1940.

THE EFFECT OF GOLD SODIUM THIOMALATE ADMINISTRATION ON THE BACTERIOSTATIC PROPERTIES OF THE SERUM IN PATIENTS WITH RHEUMATOID ARTHRITIS*

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CLINICAL reports on the use of gold salts administered parenterally for the treatment of rheumatoid arthritis are becoming more numerous, and the reported therapeutic results are more favorable. Hartfall, Garland, and Goldie¹ summarized the largest series (900 cases of all types of arthritis) in 1937. They obtained cures or striking improvement in 80 per cent of their cases of rheumatoid arthritis. The German, French, and English literature is now fairly voluminous, and, on the whole, favorably inclined toward this method of treatment, especially when confined to its use in rheumatoid arthritis.

In the United States the reports on the use of gold salts in arthritis are not as numerous. They include those by Sashin and Spanbock,² Oren,³ Phillips,⁴ and Snyder, Traeger, and Kelly.⁵ Our own experience with the use of gold salts is somewhat comparable to the favorable reports in the literature. We have observed that gold salt therapy gives a higher percentage of improvement than any form of therapy yet used by us in the treatment of rheumatoid arthritis. Our own results approximate those of Hartfall and associates to the extent that 60 per cent have shown marked improvement. We cannot at present report the percentage of cures, because it is our opinion that patients with arthritis should be followed for a minimum of five years before they are reported as cured, and none of our patients treated with gold salts have been under our observation that long.

In the past, and at present, a variety of gold compounds, in aqueous or oil solution, have been used by investigators. Our own clinical observations are limited to the use of aqueous gold sodium thiomalate, administered subcutaneously. In the usual case of rheumatoid arthritis, according to the schedule we have gradually developed, this is injected once a week in 5, 10, 25, and occasionally 50 mg. doses, until a total of 1 Gm. has been given. After an interval of twelve weeks, the entire course is repeated. It is sometimes deemed necessary to give a third course. Toxic reactions are rather frequent. Minor difficulties are encountered in at least one-half of the patients treated, and serious reactions in fully 15 per cent.

OBJECTIVE

Our objective in the investigation reported here was to study the bacteriostatic properties of the serum before and after the parenteral administration

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of gold salt in varying amounts. We were also interested in observing the effect of gold salt administration on the agglutinin titers. Such studies might throw some light on the serologic and pharmacologic action of gold salt, and suggest a hypothetical explanation of its apparent effectiveness in the treatment of rheumatoid arthritis. This and other studies now under way might also lead to a better understanding of the all too frequent toxic effects and point the way to methods of avoiding them.

MATERIALS

In planning a study of the bacteriostatic properties of serum before and after gold salt administration, an organism had to be arbitrarily selected against which to study these effects. It is generally believed that rheumatoid arthritis is a disease of infectional origin. However, the causative agent has never been conclusively demonstrated. The hemolytic streptococcus has been suggested, but on no firmer grounds than that a greater percentage of sera from patients with rheumatoid arthritis show agglutinins in high titers for hemolytic streptococci than do sera from patients with osteoarthritis, or from normal subjects.⁶ In our studies we used a beta hemolytic streptococcus (strain Greene) isolated from an acute mastoid infection. We do not in any sense imply that this organism is related to the etiology of rheumatoid arthritis. Other common laboratory microorganisms were used in various parts of the investigation. These will be detailed in the appropriate place.

The gold salt used uniformly throughout these experiments was an aqueous solution of gold sodium thiomalate administered as already outlined. It contains 50 per cent metallic gold. In addition, we were interested in observing the bacteriostatic effects following the administration of colloidal gold, a 1 per cent metallic gold in colloidal suspension, and bismuth subsalicylate, 10 per cent in oil.

The persons on whom we investigated these effects were all patients with typical rheumatoid arthritis. All of them exhibited the usual clinical and x-ray findings, together with various degrees of elevation in the sedimentation rate. Most of them were ambulatory.

TECHNIQUE FOR DETERMINING BACTERIOSTASIS

A seventeen-hour culture of hemolytic streptococcus, strain Greene (a beta strain, Group A) grown in 1 per cent dextrose meat infusion broth was diluted 1:5,000. One five-hundredth cubic centimeter (0.05 c.c.) of this dilution was added to 1 c.c. of the serum to be tested. This amount of culture usually produced from 5 to 25 colonies. The inoculated serum was then incubated at 37° C. for forty-eight hours.

The growth in the inoculated serum was estimated as follows:

A. One five-hundredth cubic centimeter (0.05 c.c.) of the inoculated serum was plated in blood (1 c.c.), agar (10 c.c.).

B. A 1:1,000 dilution of the inoculated serum was made. One five-hundredth cubic centimeter (0.05 c.c.) of this dilution was likewise plated in blood agar as above.

After twenty-four hours' incubation the colony growth on the plates was counted and the growth was estimated in colonies per cubic centimeter of original culture. This was done in the case of the undiluted serum by multiplying the number of colonies on the plate by 20, and in the case of the diluted serum, by 20,000.

All dilutions were made with stock 1 per cent dextrose meat infusion broth, dilutions with normal saline being avoided because of the known bacteriostatic effect of saline on the growth of streptococci in vitro. All tests were done in duplicate.

TABLE I
NORMAL CONTROLS
SERUM AGGLUTININ TITERS AND BACTERIOSTASIS AGAINST HEMOLYTIC STREPTOCOCCUS,
STRAIN GREENE

CASE NO.	AGGLUTININ TITER	BACTERIOSTASIS COL./C.C.
1	Negative	α 1:1,000 dilution
2	Negative	α 1:1,000 dilution
3	Negative	18,200,000
4	2+ (1:80)	32,000,000
5	Negative	14,400,000
6	4+ (1:2,560)	
	1+ (1:5,120)	α 1:1,000 dilution
7	Negative	α 1:1,000 dilution
8	Negative	4,260,000
9	Negative	1:1,000 dilution
10	Negative	44,800,000
11	Negative	α 1:1,000 dilution
12	Negative	α 1:1,000 dilution
13	Negative	α 1:1,000 dilution
14	Negative	α 1:1,000 dilution
15	Negative	α 1:1,000 dilution
16	Negative	α 1:1,000 dilution
17	2+ (1:80)	α 1:1,000 dilution
18	Negative	α 1:1,000 dilution
19	Negative	82,000,000
20	Negative	14,160,000

RESULTS

Experiment 1.—Normal controls. The sera of 20 laboratory workers, all in good health, were studied for their bacteriostatic effect on hemolytic streptococcus (strain Greene), and their agglutinin titers against this organism were determined. Bacteriostasis was negligible (Table I) except in occasional instances (Cases Nos. 3, 4, 5, 8, 10, 19, and 20). Some of these patients had recently received, or were receiving, vaccines or bacteriophage against colds. The agglutinin titers likewise were found to be about as has been usually observed in normal controls when a beta-hemolytic streptococcus was used as the antigen.

Experiment 2.—The bacteriostatic effect on hemolytic streptococcus, strain Greene, of serum taken before and after the administration of gold sodium thiomalate. The bacteriostasis was studied in patients with rheumatoid arthritis before the gold sodium thiomalate was given, and after varying amounts had been administered parenterally, in divided doses, according to the schedule suggested above for the routine treatment of these patients. It was found (Table II) that the initial bacteriostasis was about as in the normal controls.

After varying amounts of gold salt were administered there was a marked increase in the bacteriostasis in every instance, even after as little as 60 mg. had been given.

TABLE II

BACTERIOSTATIC EFFECT OF SERUM TAKEN BEFORE AND AFTER ADMINISTRATION OF GOLD SODIUM THIOMALATE

CASE NO.	GOLD SALT GIVEN BEFORE INITIAL TEST (MG.)	INITIAL BACTERIOSTASIS (COL./C.C.)	TOTAL GOLD SALT GIVEN (MG.)	FINAL BACTERIOSTASIS (COL./C.C.)
1	0	α 1:1,000 dilution	175	350
2	0	700,000	155	160
4	0	α 1:1,000 dilution	210	210
6	0	α 1:1,000 dilution	200	202
7	0	α 1:1,000 dilution	210	185
10	0	α 1:1,000 dilution	60	13,000
11	0	α 1:1,000 dilution	310	160
12	0	α 1:1,000 dilution	215	250
14	0	300,000	160	75
15	0	α 1:1,000 dilution	210	150
16	0	α 1:1,000 dilution	305	118
18	0	α 1:1,000 dilution	415	178
19	0	α 1:1,000 dilution	335	100

TABLE III

BACTERIOSTATIC EFFECT OF SERUM TAKEN AT VARIOUS INTERVALS DURING THE COURSE OF GOLD SODIUM THIOMALATE ADMINISTRATION

CASE NO.	GOLD SALT GIVEN BEFORE INITIAL TEST (MG.)	INITIAL BACTERIOSTASIS (COL./C.C.)	TOTAL GOLD SALT GIVEN (MG.)	FINAL BACTERIOSTASIS (COL./C.C.)
3	15	α 1:1,000 dilution	280	60
5	250	Unsatisfactory	900	15
8	85	α 1:1,000 dilution	147	112
9	275	320	425	70
13	15	Unsatisfactory	215	250
17	205	50	250	50
20	240	110	921	55

Table III reports the bacteriostatic properties of the serum in intermediate stages of the administration of gold salt. In these subjects, where gold salt had been given before the initial tests were done, the initial bacteriostasis was greater than normal (except in Cases 3 and 8, where only 15 and 85 mg. had been given) and was increased after more gold salts had been administered subsequent to the initial determination. It will be observed that the bacteriostasis indeed was roughly proportional to the amount of gold salt given. In general, the maximum bacteriostasis seen in these experiments was observed after 147 to 155 mg. had been given, and it did not materially increase even after many times these amounts of gold had been administered.

Experiment 3.—We were interested in observing the effect of gold sodium thiomalate on the agglutinin titers of the serum against hemolytic streptococcus (strain Greene). Initial determinations were done and repeated after varying amounts of gold salt had been given. In general, no consistent changes were observed (Table IV).

TABLE IV

SERUM AGGLUTININ DETERMINATIONS BEFORE AND AFTER THE ADMINISTRATION OF GOLD SODIUM THIOMALATE

CASE NO.	GOLD SALT GIVEN BEFORE INITIAL TEST (MG.)	INITIAL AGGLUTININ TITER	TOTAL GOLD SALT GIVEN (MG.)	FINAL AGGLUTININ TITER
1	0	4+ (1:40)	175	3+ (1:20)
2	0	4+ (1:160) 1+ (1:2,560)	155	Negative
3	15	4+ (1:160) 1+ (1:2,560)	286	3+ (1:320)
4	0	Negative	210	Negative
5	250	Negative	900	Negative
6	0	Negative	200	Negative
7	0	3+ (1:80) 1+ (1:160)	210	4+ (1:2,560)
8	85	Negative	147	Negative
9	275	4+ (1:320) 1+ (1:1,280)	425	2+ (1:160)
10	0	Negative	60	Negative
11	0	Negative	310	Negative
12	0	Negative	245	Negative
13	15	Negative	215	Negative
14	0	Negative	160	Negative
15	0	Negative	210	Negative
16	0	Negative	305	Negative
17	205	Negative	250	Negative
18	0	Negative	415	Negative
19	0	Negative	335	Negative
20	260	4+ (1:20) 1+ (1:80)	921	Negative

TABLE V

EFFECT OF CONTINUING ADMINISTRATION OF GOLD SODIUM THIOMALATE ON THE BACTERIOSTATIC POWER OF THE SERUM

CASE NO.	GOLD SALT (MG.)	BACTERIOSTASIS (COL./C.C.)
2	0	700,000*
	50	1,600,000
	115	5,000
	155	160
3	15	\propto 1:1,000 dilution
	119	10,150
	286	60
4	0	\propto 1:1,000 dilution
	60	21,000
	210	210
11	0	\propto 1:1,000 dilution
	85	10,000
	310	160
18	0	\propto 1:1,000 dilution
	115	17,800
	415	178

*Vaccines previously administered.

Experiment 4.—The effect of the continuing administration of gold salt on the bacteriostatic power of the serum. The subjects received weekly injections of gold sodium thiomalate, and the bacteriostasis was determined after varying amounts had been given. From a review of Table V, it is observed that the bacteriostasis in each case increased in rough proportion to the total

amount of gold salt given. In Case 2, for reasons not obvious, the original bacteriostasis was greater than usual in the controls; and after 50 mg. of gold salt, it was less than at the initial determination. After 115 mg., however, marked bacteriostasis was found, and after 155 mg. the bacteriostasis was still more pronounced.

TABLE VI

CHANGES IN THE BACTERIOSTATIC POWER OF THE SERUM AFTER STOPPING ADMINISTRATION OF GOLD SODIUM THIOMALATE

CASE NO.	DATE	AMOUNT OF GOLD SALT GIVEN (MG.)	BACTERIOSTASIS (COL./C.C.)
6	8/24/39	0	α 1:1,000 dilution
	9/21/39	125	2,024
	10/31/39	200	202
	Injections of gold salts stopped		
	2/13/40	0	60,000,000
11	9/19/39	0	α 1:1,000 dilution
	10/21/39	310	160
	Injections of gold salts stopped		
	4/ 4/40		α 1:1,000 dilution
20	6/28/39	260	110
	11/20/39	921	55
	Injections of gold salts stopped		
	2/15/40		142,000,000

Experiment 5.—Changes in the bacteriostatic power of the serum after stopping the administration of gold sodium thiomalate. In this experiment, 3 patients with rheumatoid arthritis received 200, 310, and 921 mg., respectively, of the gold salt in divided weekly doses over varying lengths of time. Marked bacteriostasis resulted. In each case the administration was then stopped for important clinical reasons. After varying intervals of time had elapsed, the bacteriostasis was again determined and was found to have returned to the levels usually found in normal controls. In other words, after the gold salt had been withheld from these patients for from three to six months, the previously observed bacteriostatic power of their serum was found to be absent (Table VI).

TABLE VII

EFFECT OF COLLOIDAL GOLD ADMINISTRATION ON THE BACTERIOSTATIC POWER OF THE SERUM

CASE NO.	COLLOIDAL GOLD GIVEN BEFORE INITIAL TEST	INITIAL BACTERIOSTASIS (COL./C.C.)	TOTAL COLLOIDAL GOLD GIVEN (C.C.)	FINAL BACTERIOSTASIS
101	0	α 1:1,000 dilution	6.0	α 1:1,000 dilution
102	0	α 1:1,000 dilution	14.8	α 1:1,000 dilution
103	0	α 1:1,000 dilution	11.5	α 1:1,000 dilution
104	0	3,000,000	3.6	α 1:1,000 dilution

Agglutinin titers show no change throughout.

Experiment 6.—Effect of colloidal gold administration on the bacteriostatic power of the serum. We were curious to see whether preparations of colloidal gold would induce bacteriostasis, as was observed after the administration of gold sodium thiomalate. In 4 patients a preparation of 1 per cent colloidal gold was administered parenterally in divided weekly doses. In

Case 102 a total of 14.8 c.c. was given. This latter was equivalent to 280 mg. of gold sodium thiomalate in terms of metallic gold content. From Table VII, it is obvious that no bacteriostasis was induced in this case or in any other case under observation.

Experiment 7.—Bacteriostasis before and after the parenteral administration of bismuth subsalicylate, 10 per cent in oil, in divided weekly doses. The initial bacteriostasis was determined, and again after varying amounts of bismuth had been administered. Some bacteriostasis was produced by this method, but not in amounts comparable with that following the administration of gold salt (Table VIII).

TABLE VIII
EFFECT OF BISMUTH ADMINISTRATION ON THE BACTERIOSTATIC
POWER OF THE SERUM

CASE NO.	BISMUTH GIVEN BEFORE INITIAL TEST	INITIAL BACTERIOSTASIS	TOTAL BISMUTH GIVEN (C.C.)	FINAL BACTERIOSTASIS (COL./C.C.)
201	0	\propto 1:1,000 dilution	9.0	93,750
202	0	\propto 1:1,000 dilution	15.0	\propto 1:1,000 dilution
203	0	\propto 1:1,000 dilution	17.5	25,600,000
204	0	\propto 1:1,000 dilution	11.5	\propto 1:1,000 dilution
205	0	\propto 1:1,000 dilution	14.0	40,000,000
206	0	\propto 1:1,000 dilution	13.5	30,000,000
207	0	\propto 1:1,000 dilution	8.5	\propto 1:1,000 dilution
208	0	\propto 1:1,000 dilution	11.0	3,000,000

TABLE IX
IN VITRO EFFECT OF GOLD SODIUM THIOMALATE ON THE GROWTH OF HEMOLYTIC
STREPTOCOCCUS, STRAIN GREENE

DILUTIONS OF GOLD SALT (MG./C.C.)	DEVELOPMENT OF PRECIPITATE AFTER ADDITION OF CULTURE	GROWTH OF HEMOLYTIC STREPTOCOCCUS, STRAIN GREENE, AFTER 48 HOURS' INCUBATION (COL./C.C.)	
		EXPERIMENT 11	EXPERIMENT 12
10.0	0	Sterile	Sterile
1.0	1 plus	Sterile	Sterile
0.1	0	Sterile	Sterile
0.01	0	Sterile	Sterile
0.001	0	Sterile	Sterile
0.0001	0	Sterile	Sterile
0.00001	0	300,000	5,720
0.000001	0	\propto 1:1,000	\propto 1:1,000
0.0000001	0	\propto 1:1,000	\propto 1:1,000

Experiment 8.—The in vitro effect of varying dilutions of gold sodium thiomalate on the growth of hemolytic streptococcus, strain Greene. Varying dilutions of gold sodium thiomalate in 1 per cent dextrose beef infusion broth were prepared. The first tube contained 10 mg. of gold sodium thiomalate in 1 c.c., and the others contained lesser amounts, as recorded in Table IX. To each dilution was added 0.05 c.c. of 1:5,000 dilution of an eighteen-hour culture of hemolytic streptococcus (strain Greene). After forty-eight hours' incubation the amount of growth was determined.

This experiment was repeated many times with rather consistent findings. Two typical results are reported in Table IX. In general, it was observed

that there was no growth in the tubes containing from 10 to 0.0001 mg. of gold sodium thiomalate, after which moderate and then luxuriant growth was observed.

Six other organisms were arbitrarily selected and subjected to identical in vitro studies. The results were similar to those obtained when using hemolytic streptococcus, strain Greene, although less susceptibility to gold was shown by some of these organisms. The amount of bacteriostasis was roughly proportional to the concentration of gold salt present in the dilution. The details of these findings are summarized in Table X.

TABLE X

IN VITRO EFFECT OF VARYING DILUTIONS OF GOLD SODIUM THIOMALATE ON THE GROWTH OF OTHER COMMON LABORATORY ORGANISMS

DILUTIONS OF GOLD SALT (MG./C.C.)	BACTERIAL GROWTH AFTER 48 HOURS' INCUBATION (COL./C.C.)					
	STAPH. ALBUS ¹	STAPH. AUREUS ²	STREP. VIRIDANS ³	STREP. NON-HEMOLYTIC ⁴	PNEUMO. TYPE III ⁵	PNEUMO. TYPE VII ⁶
10.0	0	0	0	0	0	0
1.0	0	0	0	0	0	0
0.1	400,000	0	600,000	0	300	0
0.01	8,600,000	0	α 1:1,000	340,000	8,640	6,200
0.001	α 1:1,000	2,600,000	α 1:1,000	2,800,000	4,280,000	140,000
0.0001	α 1:1,000	10,040,000	α 1:1,000	α 1:1,000	α 1:1,000	8,000,000
0.00001	α 1:1,000	α 1:1,000	α 1:1,000	α 1:1,000	α 1:1,000	α 1:1,000
0.000001	α 1:1,000	α 1:1,000	α 1:1,000	α 1:1,000	α 1:1,000	α 1:1,000
0.0000001	α 1:1,000	α 1:1,000	α 1:1,000	α 1:1,000	α 1:1,000	α 1:1,000

¹Staphylococcus albus (isolated from a boil).

²Staphylococcus aureus hemolytic (isolated from a blood stream infection).

³Staphylococcus viridans (isolated from a patient with endocarditis).

⁴Nonhemolytic streptococcus (isolated by B. J. Clawson and others from a patient with "chronic arthritis").

⁵Pneumococcus type III (from sputum).

⁶Pneumococcus type VII (from sputum).

TABLE XI

IN VITRO EFFECT OF VARYING DILUTIONS OF GOLD SODIUM THIOMALATE IN 50 PER CENT WHOLE HUMAN BLOOD ON THE GROWTH OF HEMOLYTIC STREPTOCOCCUS, STRAIN GREENE

DILUTIONS OF GOLD SALT (MG./C.C.)	GROWTH OF HEMOLYTIC STREPTOCOCCUS, STRAIN GREENE, AFTER 48 HOURS' INCUBATION	
	COL./C.C. EXPERIMENT 1	COL./C.C. EXPERIMENT 2
10.0	7,720	2,550
1.0	350,000	220,000
0.1	α 1:1,000	α 1:1,000
0.01	α 1:1,000	α 1:1,000
0.001	α 1:1,000	α 1:1,000
0.0001	α 1:1,000	α 1:1,000
0.00001	α 1:1,000	α 1:1,000
0.000001	α 1:1,000	α 1:1,000
0.0000001	α 1:1,000	α 1:1,000

When whole blood was added to the extent of 50 per cent of the final dilutions in these in vitro studies, the bacteriostatic power of the gold salt was destroyed in all except the first two tubes (Table XI).

DISCUSSION

In 1890 Koch,⁷ and later DeWitt and Sherman,⁸ reported that gold cyanide in dilutions of 1:2,000,000 inhibited the in vitro growth of tubercle bacilli.

Shortly after this, White⁹ reported favorable results following the hypodermic administration of a combination of gold and manganese in the treatment of tuberculosis. Somewhat later, Mayer,¹⁰ Spiess and Feldt,¹¹ Mollgaard,¹² and others reported on this form of therapy in tuberculosis. In 1928-1929 Umber¹³ in Germany and Forestier¹⁴ in France almost simultaneously applied the use of gold salts to the treatment of rheumatoid arthritis, because of the hypothetical association of rheumatoid arthritis and tuberculosis.

One explanation easily suggested for the apparent effectiveness of gold salts administered parenterally in certain infections was that they acted in the body by exerting a direct bacteriostatic effect. Since, however, the *in vitro* inhibition of the growth of tubercle bacilli by gold salts was destroyed in these early experiments by the addition of human or animal blood, it was felt that the favorable results were not due to a direct bacteriostatic effect, but to the stimulation of some unexplained defense mechanism in the patient, most probably the reticulo-endothelial system. Our own observations also showed the *in vitro* loss of bacteriostatic effect when blood was added to the broth dilutions, due possibly to a precipitation of the gold to an insoluble and inert form. Such inactivation may not occur, however, when the salts are injected parenterally.

Certainly there is no evidence that gold salts produced bacteriostasis by stimulating any of the known defense mechanisms of the body. Our observations show that the agglutinin titers, at least for the particular streptococcus used by us, are not consistently affected, and the results of at least a thousand white and differential blood counts we performed, but have not reported here, do not suggest that the leucocyte pattern is altered, except for an occasional increase in the eosinophiles, during the entire course of gold therapy. Also the bacteriostasis is not maintained, as might be expected from a stimulation of the protective mechanisms, but is rapidly lost after the cessation of gold salt administration.

The evidence induced by these experiments, therefore, supports the theory that gold salts could act by a direct effect on the causative agent of the rheumatoid arthritis, assuming that the causative agent is infectious. Such a mechanism now appears to be the case after the administration of sulfanilamide and allied compounds. In what way this would take place, by coagulation of the bacterial proteins, interfering with the processes of oxidation and reduction of bacterial metabolism, or by actual dissolution of the organism, is, of course, purely hypothetical; as a matter of fact, it is equally hypothetical at present in the case of sulfanilamide and most other forms of chemotherapy.

Still another mode of action of gold salts has been suggested. In experimental tuberculosis it was considered by some investigators that gold owes its effects to a toxic action on the capillary endothelium. Hyperemia, hemorrhage, and edema have been demonstrated about the tuberculous lesions. Also gold was found to be more toxic for tuberculous animals than for the controls. Whether similar phenomena take place in rheumatoid arthritis has not been determined.

An understanding of the mode of action of gold salts is important, since it might carry with it a knowledge of the kind of patients in which gold is

likely to succeed, a rational system of dosage, an explanation of the failures, and an indication of the lines which might profitably be followed in the development of new compounds. It is well known in chemotherapy that the slightest modification of a molecule carries with it profound changes in curative or toxic properties. This has been especially demonstrated in the past in the development of the pentavalent arsenic series.

Also the intermediate and final fate of gold salts injected parenterally must be thoroughly investigated. However, until an adequate method for the quantitative determination of gold salts and their derivatives in the blood, organs, and excreta, is at hand, knowledge concerning their absorption, distribution, and excretion will be lacking, and a rational system for administration cannot be developed. This latter knowledge is of special importance, in view of the toxic effects so commonly sequential to gold therapy.

CONCLUSIONS

1. Subcutaneous injections of gold sodium thiomalate administered to rheumatoid arthritis patients in divided doses totaling as little as 60 mg. and up to 921 mg. were followed by a marked increase in the bacteriostatic power of the serum against hemolytic streptococcus, strain Greene.

2. The observed bacteriostatic effects were in rough proportion to the total amount of gold sodium thiomalate given; the maximum effect being attained after from 147 to 155 mg. had been administered. The bacteriostasis disappeared after stopping the administration for three to six months.

3. Parenteral injections of gold sodium thiomalate did not significantly increase or decrease the agglutinin titers for hemolytic streptococcus, strain Greene.

4. Colloidal gold in amounts administered by us did not produce significant bacteriostasis. Bismuth subsalicylate was hardly more effective.

5. Gold sodium thiomalate was found to be bacteriocidal in vitro against streptococcus hemolytic strain Greene, in dilutions through 0.000001 per cent, and bacteriostatic in higher dilutions. Other common laboratory organisms were similarly, though not as markedly, affected. The in vitro bacteriostasis was roughly proportional to the concentration of gold salt. The addition of whole blood to the extent of 50 per cent of the final dilutions in these in vitro studies almost completely destroyed the bacteriostatic effect.

REFERENCES

1. Hartfall, S. J., Garland, H. G., and Goldie, W.: Gold Treatment of Arthritis, A Review of 900 Cases, *Lancet* 2: 784, 838, 1937.
2. Sashin, D., and Spanbock, J.: Intravenous Gold Therapy in Rheumatoid Arthritis, *Med. Rec.* 145: 429, 1937.
3. Oren, H.: Arthritis and Its Treatment With Gold Salts, *J. M. Soc. New Jersey* 33: 591, 1936.
4. Phillips, R. T.: The Treatment of Arthritis With Gold Salts, *New England J. Med.* 214: 114, 1936.
5. Snyder, R. G., Traeger, C., Kelly, L.: Gold Therapy in Arthritis; Observations on 100 Cases Treated With Gold Sodium Thiosulphate and Aurocein, *Ann. Int. Med.* 12: 1672, 1939.

6. Hartung, E. F., and Neil, M. S.: Precipitins for *Streptococcus Hemolyticus* in Rheumatoid Arthritis Serums, *J. LAB. & CLIN. MED.* 22: 881, 1937.
7. Koch, R.: Ueber bakteriologische Forschung, *Deutsche med. Wehnschr.* 16: 756, 1890.
8. DeWitt, L. M., and Sherman, H.: Tuberculocidal Action of Certain Chemical Disinfectants, *J. Infect. Dis.* 15: 245, 1914.
9. White, J. B.: Tubercular Diathesis Controlled by Gold and Manganese in Combination, *Am. Med. Surg. Bull.* 7: 388, 1894.
10. Mayer, A.: Zur Chemotherapie der Lungentuberkulose, *Deutsche med. Wehnschr.* 39: 1678, 1913; *Beitr. z. Klin. d. Tuberk.* 32: 211, 1914.
11. Spiess, G., and Feldt, A.: Tuberkulose und Goldkantharidin, mit besonderer Berücksichtigung der Kehlkopf tuberkulose, *Deutsche med. Wehnschr.* 40: 579, 1914.
12. Spiess, G.: Die intravenöse Behandlung der Tuberkulose mit Goldkantharidin, *München. med. Wehnschr.* 41: 842, 1914.
13. Møllgaard, H.: Chemotherapy of Tuberculosis, Copenhagen, 1924.
14. Umber, F.: Zur Diagnostik und Therapie chronischer Gelenkerkrankungen, *Med. Welt* 3: 593, 1929.
15. Forestier, J.: L'aurothérapie dans les rhumatismes chroniques, *Bull. et mém. Soc. méd. d. hôp. de Paris* 53: 323, 1929.

580 PARK AVENUE

TREATMENT OF PNEUMONIA IN RATS*

A COMPARATIVE STUDY OF THE THERAPEUTIC EFFICIENCY OF SULFAPYRIDINE AND RABBIT SERUM, AND COMBINATIONS OF THE TWO, IN ARTIFICIALLY INDUCED TYPE I PNEUMOCOCCUS INFECTION OF ALBINO RATS

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GROSS and Cooper¹ in 1937 treated type I pneumococcal infections in rats with sulfanilamide and rabbit antipneumococcus serum, alone and in combination. They concluded, from their experiments, that sulfanilamide was better than serum and that combined therapy was more effective than either therapy alone.

In 1939, Kepl and Gunn² similarly treated type I pneumococcal infections in rats with sulfapyridine and rabbit serum. They concluded that, when treatment was begun within four hours after inoculation, the combined use of serum and sulfapyridine was more effective than either alone. But, when infection was well established, serum was more efficacious than sulfapyridine; while at this stage the combined use of these preparations offered no more than serum alone.

Both groups of workers inoculated the animals intrabronchially with type I pneumococci suspended in gastric mucin. Neither, however, report on blood cultures prior to and during treatment of the animals, making it difficult, therefore, to evaluate accurately the effectiveness of any therapy used, since the presence of infection had not been definitely established.

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The experiments we report here were done to determine the comparative effectiveness of sulfapyridine and type I rabbit antipneumococcus serum when used alone and in combination in the treatment of type I pneumococcal infection in rats.

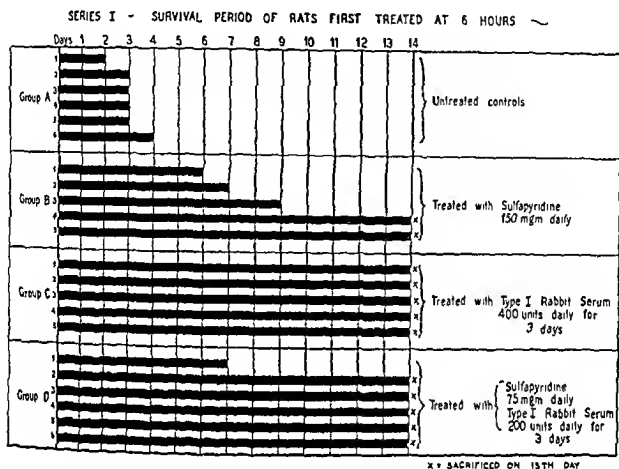


FIG. 1.

METHODS

Inoculation of the rats, as described in a previous paper,³ was done by spraying a pneumococcal culture into both the pharynx and nares. This culture, which was suspended in 15 c.c. of a physiologic solution of sodium chloride, consisted of the washings of the peritoneal cavity of a white mouse infected with type I pneumococci (RI strain), and contained, as a result of the peritoneal inflammation, natural mucin in addition to the other components of an inflammatory exudate. The procedure was entirely nontraumatizing.

To insure accurate dosage, the sulfapyridine was suspended in diluted condensed milk and was introduced through a stomach tube. The serum was administered by intraperitoneal injection.

A total of 67 rats, weighing approximately 300 Gm. each, were thus inoculated. Fifty-six became infected, as manifested by the development of positive blood cultures. Cultures of the blood were made prior to treatment, at six hours and twenty-four hours after inoculation, and were repeated at twenty-four-hour intervals, until spontaneous death occurred or recovery ensued. Only those animals of the control and treated series which developed blood stream invasion were included in this report.

The 56 rats which became infected were separated into two series, each of which, in turn, was divided into four groups.

In Series I, consisting of 22 rats, treatment was started six hours after inoculation, and blood for culture was taken just prior to beginning treatment. Included in this series were: Group A, 6 untreated controls. Group B, 5 animals which were treated with seven doses each of 150 mg. of sulfapyridine at twenty-four-hour intervals. Group C, 5 animals which were treated with three doses each of 400 units of type I rabbit antipneumococcus serum at twenty-four-hour intervals. Group D, 6 animals which were treated with three doses each of 200 units of type I antipneumococcus serum at twenty-four-hour intervals and seven doses each of 75 mg. of sulfapyridine at twenty-four-hour intervals.

In Series II, consisting of 34 rats, treatment was started twenty-four hours after inoculation. Blood cultures were made prior to institution of treatment at six and twenty-four hours after inoculation. Included in this series were: Group E, 8 untreated controls. Group F, 8 animals which were treated with three doses each of 225 mg. of sulfapyridine, at intervals of twenty-four hours. Group G, 9 animals which were treated with three doses each of 600 units of type I antipneumococcus serum at twenty-four-hour intervals. Group H, 9 animals which were treated with three doses each of 300 units of type I antipneumococcus serum at twenty-four-hour intervals and seven doses each of 150 mg. of sulfapyridine at twenty-four-hour intervals.

RESULTS

Series I (Treatment begun at six hours). Group A: All 6 untreated controls died, the mortality rate being 100 per cent. The average period of survival was three days. Group B (Sulfapyridine): Three of the 5 rats died the mortality rate being 60 per cent. The average period of survival of those dying spontaneously was 7.6 days. Group C (Serum): All five rats recovered the mortality rate was zero per cent. Group D (Sulfapyridine and serum): One of the 6 rats died on the seventh day; the mortality rate was 16.6 per cent.

Series II (Treatment begun at twenty-four hours). Group E: Seven of the 7 untreated controls died spontaneously, the mortality rate being 86 per cent. The average period of survival was three days. Group F (Sulfapyridine): Five of the 8 rats died; the mortality rate was 62.5 per cent. The average survival period of those dying spontaneously was 5.6 days. Group G (Serum): Two of the 9 rats died, the mortality rate being 22.2 per cent. The average period of survival of those dying spontaneously was five days. Group H (Sulfapyridine and serum): Two of the 9 rats died; the mortality rate was 22.2 per cent. The average survival period of those dying spontaneously was 4.5 days.

In the animals that recovered, the blood cultures, on the average, became negative on the fourth day of sulfapyridine therapy, on the third day of serum therapy, and on the fourth day of serum and sulfapyridine therapy.

Post-mortem Results.—All rats which survived were killed at the end of the fourteenth day and autopsied. The results of the post-mortem examination confirmed the existence of previous infection.

Of the 26 rats that died spontaneously, in both the control and treated groups, it was found that 6 (A1, E2, E7, F1, F4, and F5) had pneumonia and

SERIES II - SURVIVAL PERIOD OF RATS FIRST TREATED AT 24 HOURS

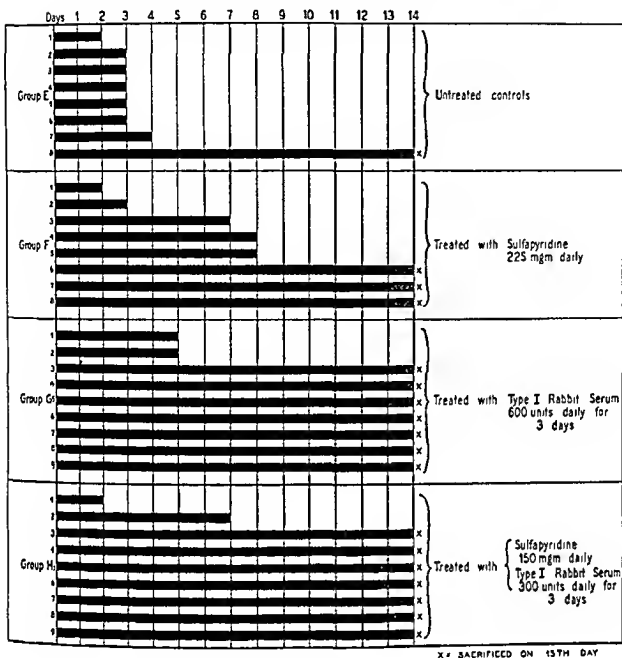


Fig. 2.

% OF SURVIVALS AND MORTALITY RATES OF RATS INFECTED WITH TYPE I PNEUMOCOCCI

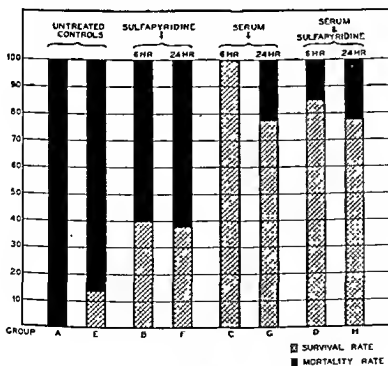


Fig. 3.

empyema; 5 (A2, A4, E3, E6, and H2) had pneumonia, empyema, and pericarditis; 3 (A6, B2, and D1) had pneumonia and pericarditis; 2 (E1, F3) had pneumonia alone; 2 (G1, G2) had pneumonia, pericarditis, and meningitis; 2 (A3, E4) had pneumonia, pericarditis, and peritonitis; 2 (B1, F1) had bilateral empyema; 1 (A5) had pericarditis alone; 1 (B3) had meningitis alone; 1 (E5) had meningitis, empyema, and peritonitis; and 1 (H1) presented no lesions.

SUMMARY AND CONCLUSIONS

1. Type I pneumococcal infection, as manifested by positive blood cultures, was produced in 56 albino rats. Sulfapyridine alone, type I rabbit antipneumococcus serum alone, or sulfapyridine and serum in combination, were administered to 42 animals, treatment having been started at either six hours or twenty-four hours after inoculation.

2. In the treatment of pneumonia in rats, type-specific serum appeared to be more efficacious than sulfapyridine alone; whereas a combination of serum and sulfapyridine in approximately one-half the dosage of either used separately gave no better results than serum without sulfapyridine.

We wish to express our appreciation to Doctors Tasker Howard, J. Hamilton Crawford, Wade W. Oliver, and Arnold Eggerth for their aid and criticism in the preparation of this paper, and to Misses Anne Smith and Dora Zuckerman for their aid in the preparation of the bacteriologic material used.

REFERENCES

1. Gross, P., and Cooper, F. B.: P-Aminobenzene-sulfonamide and Antipneumococcal Serum Therapy in Type I Pneumococcal Infections of Rats, *Proc. Soc. Exper. Biol. & Med.* 36: 535, 1937.
2. Kepl, M., and Gunn, F. D.: Sulfapyridine and Serum Therapy in Experimental Lobar Pneumonia of Rats, *Proc. Soc. Exper. Biol. & Med.* 40: 529, 1932.
3. Loughlin, E. H., Spitz, S. H., and Bennett, R. H.: A Simple Nontraumatizing Method of Inducing Pneumococcal Infection in Albino Rats, *J. LAB. & CLIN. MED.* 26: 1181, 1941.

CHROMOPHOBE PITUITARY ADENOMA WITH SIMMONDS' DISEASE*

CASE REPORT WITH AUTOPSY

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ANTERIOR pituitary tumors causing a clinical and pathologic pattern resembling Simmonds' disease (pituitary cachexia) are of sufficient rarity to justify the report of a single case.

The present report deals with the post-mortem finding of a large cystic chromophobe adenoma of the anterior pituitary body in a middle-aged negress, and serves to explain a train of mental, visual, and cachectic disturbances noted clinically.

The knowledge regarding Simmonds' disease has increased considerably since Simmonds¹ first report in 1914. A helpful summary of the outstanding pathologic and clinical characteristics of the condition, as accepted today, was given in 1938 by Farquharson, Belt, and Duff² who also reported one of the largest personal series of cases.

Excellent reviews of the literature were made by Calder in 1932³ and Silver in 1933.⁴ Of the 70 cases from the world's literature collected by Calder and accepted by him as true examples of Simmonds' disease, only 9 were due to tumors or cysts. Several other writers have mentioned the rarity of cases of Simmonds' disease due to neoplasm, although Horrax,⁵ in speaking of material encountered in a neurosurgical clinic, stated that "cases of tumor exhibiting evidences of Simmonds' disease . . . are not particularly infrequent."

REPORT OF CASE

History.—M. G., aged 54 years, a widowed negress and a domestic by occupation, was admitted to the Mental Department of the Pittsburgh City Home and Hospitals on July 9, 1936. Her complaints at this time were sleepiness, nervousness, weakness, lack of energy and interest, and inability to work.

Married and widowed, she had had one pregnancy that resulted in miscarriage. In 1922 her uterus and adnexa had been removed for fibromyomas, chronic salpingitis, and ovarian cysts. Her normal weight was 118 pounds.

She had been employed and in good health until the spring of 1934, when she began to notice abnormal sleepiness. Sometimes she would sleep continuously for as long as sixteen or seventeen hours. She also noticed pain in her back, hips, and legs.

During the following year she had several "spells," in which everything "went black." During these attacks she stated that she lost consciousness, but had no convulsions and no urinary incontinence.

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In March, 1936, she visited the outpatients clinic of a Pittsburgh hospital with the above complaint. Her physical examination was reported negative at this time. Blood pressure was 100/70. Urinalysis was negative. Blood Wassermann and Kahn tests were negative. Blood sugar was 100 mg. Blood urea was 16.6 mg., fasting blood sugar was 84 mg. per 100 cc. She made no further visits to this clinic.

Between this time and her present admission she complained of seeing "bugs on blankets" and "pygmies in corners," both of which were apparently visual hallucinations.

Because of her queer behavior her sister summoned a city physician in July, 1936. This physician recommended her admission to a mental hospital. He noted "low blood pressure, general asthenic symptoms of neurasthenia bordering on melancholia, and unwillingness to eat."

During her twenty-day stay in the observation ward, the patient was listless and quiet. She was cooperative, but often nervous and depressed. She was always sleepy. During an interview she fell asleep in her chair several times. She complained of numbness and weakness in her arms and legs, and lack of appetite. She stated that she had been eating only one meal daily while at home. She remembered the visual hallucinations but experienced them no more. Her general knowledge was poor. She was coherent and relevant in her answers. Her emotional reaction was simple and childish. Her orientation was correct and her memory was good. She was not delirious.

The family history was irrelevant except that one sister had died of a mental disease of unknown classification.

Physical Examination.—The patient was a well-developed mulatto negress, weighing 101 pounds. The upper jaw was edentulous; the lower teeth were carious. The pupils reacted sluggishly to light. The chest was clear. The blood pressure was 120/64. Pulse rate was 82 per minute. The temperature was 99.6° F. The abdomen was soft, with wrinkling of the skin. There was an old midline abdominal scar. Reflexes were noted as normal at this time. No other significant findings were recorded. Blood Hinton, Kahn, and Wassermann tests were negative. Urinalysis was negative.

Progress.—The patient was brought before the committing staff on the tenth day of hospitalization. She was not committed but was transferred to the Home Department, with a diagnosis of mental deficiency and dental caries.

In the Home Department she attracted little notice. She lost weight gradually, failed in strength, and had increased difficulty in swallowing. She was apathetic and mentally dull. Her sight failed gradually. She helped with the work around the ward until prevented by her increasing weakness. She became bedridden and helpless in September, 1937. On October 10, 1937, she had an epileptiform convulsion. From this point on she failed rapidly. She was almost blind in November. On November 9, 1937, she passed into a semicomatose state from which she never recovered. On December 2, 1937, she had three epileptiform seizures. On December 7, 1937, she died.

Clinical Diagnosis.—Cachexia of unknown origin.

Autopsy.—Autopsy was performed three hours after death.

The body was extremely emaciated, weighing only 75 pounds. The scalp hair was of normal amount, black, and kinky. The skin of face was loose and wrinkled, as was the skin of the abdomen. The breasts were flat and atrophic. The abdomen presented many old white striae. There was an old healed 15 cm. midline scar below the umbilicus. Pubic and axillary hair were scanty. The escutcheon was feminine. There were many dependent pressure sores, including ones in the scapular, trochanteric, and sacral regions. There were also sores over either external malleolus. There was dependent edema of both lower legs.

The pupils were round, regular, and equal, each measuring 0.4 cm. in diameter. The upper jaw was edentulous; the teeth remaining in the lower jaw were carious. The lower jaw was prominent, long, and protruding. The chest was flat and symmetrical. The abdomen was soft and flat.

Head.—On attempting to remove the brain, a large partially cystic tumor mass was discovered in the sella turcica, extending up into the base of the brain. The brain was removed without rupture of the cystic portions of the tumor. The solid portion of the tumor

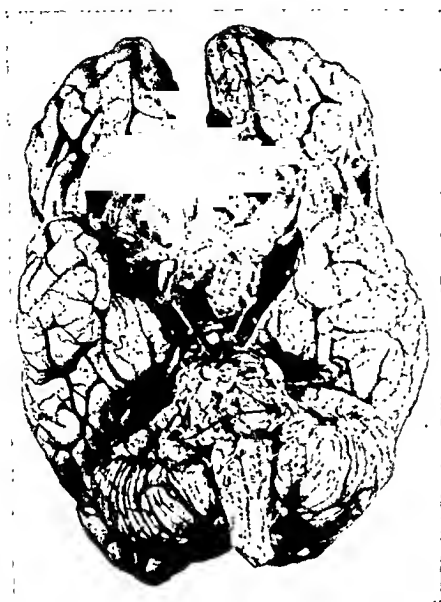


FIG. 1.—Showing tumor at base of brain. The cystic lobes are seen on either side



FIG. 2.—Sagittal section of the cerebrum showing the solid portion of the tumor pressing against the fornix and corpus callosum.

had to be removed forcibly from the right side of the sella. At this point part of the mushy, gelatinous tumor substance was left behind, where it had eroded through the pituitary capsule and adjacent dura, and grown around the right internal carotid artery in the cavernous sinus. The floor of the sella was thinned, but its continuity was unbroken. The sella was 2.25 cm. long, 2.50 cm. wide, and 2 cm. deep. Except for the sellar enlargement, the base of the skull was not remarkable.

Brain.—The brain weighed 1,400 Gm. and measured 17.75 by 14 by 9.5 cm. The tumor mass at the base of the brain was 10 cm. wide, 6 cm. long, and 6.5 cm. in depth (see Fig. 1). The tumor was composed of a solid sellar portion and two symmetrical cystic prolongations, one extending into either cerebral hemisphere.

The solid sellar portion presented a flat, yellowish-gray, soft but firm, granular, and friable cut surface.

The cystic prolongations were remarkable in their symmetry, large size, and encroachment upon frontal and temporal lobes. Each of these two cystic units measured 5 by 4.75 by 4.50 cm. Sagittal section of the cerebrum passed through the septum, dividing the two cysts so that neither was ruptured by the cut. The cyst walls were thin and fragile, with a dull bluish translucence. Each was filled with brown finely flocculent fluid. The extension of the cysts into brain substance was anterolateral in either case. On the left side the bluish translucency of the cyst could be seen externally in the divulged lateral fissure in the frontal lobe.

No actual invasion of the brain tissue was observed, but there was compression atrophy of both frontal and temporal lobes, with flattening of the convolutions and near obliteration of the sulci over the vertex.

The arterial and nervous elements at the base of the brain were greatly distorted. The basilar artery and the vertebral arteries were thin and attenuated. The divisional branches of the basilar artery gave off thin communicating branches to the internal carotid arteries on either side of the tumor. The anterior cerebral arteries could not be identified. The optic nerves were stretched, thinned, and flattened. The cystic prolongations reached anteriorly to the tip of either frontal lobe and extended back along the corpus callosum to the plane of the mammillary bodies (Fig. 2). The solid portion of the tumor extended from the sella up to the fornix where it had caused thinning of the fornix and corpus callosum. The solid tumor nearest the fornix was necrotic, being reddish-brown, mushy, and granular.

Microscopic Description.—From formalin-fixed tissue paraffin sections were made and stained with hematoxylin and eosin, phosphotungstic acid-hematoxylin, van Gieson's connective tissue method, Wilder's reticulum stain, and Spark's differential stain for pituitary cells.

The tumor was predominantly chromophobic. Small, slightly elongated or polyhedral cells were arranged usually in compact solid sheets and cords (Fig. 3), but often grouped around tiny blood vessels to form pseudorosettes (Fig. 4). A scanty, irregular fibrous connective tissue stroma was present. The essential cells were of two types (Fig. 5). The commoner type was large and vesicular, with a heavy chromatin network and moderate variation in size and shape. The other type was smaller, with a compact, almost solidly staining chromatin content. There were very occasional normal mitotic figures. The cytoplasm of most cells was scanty, pale, slightly granular, and neutrophilic. In the phosphotungstic acid-hematoxylin stains numerous, fine, rodlike mitochondria were found distributed chiefly in the periphery of the cytoplasm. In one small area a few large eosinophilic cells were seen. Near these eosinophilic remnants was a tiny microscopic colloid cyst, similar to the cysts found in the normal pars intermedia.

The cyst walls were composed of fibrous tissue in whose meshes were many columns of tumor cells, similar to those in solid portions of the growth. Smear of the cyst fluid showed numerous red blood cells and an occasional tumor cell.

Neck.—Unfortunately the thyroid gland was not examined.

Thorax.—The left lung weighed 160 Gm., the right lung 270 Gm. The heart was very small, weighing only 135 Gm.

Abdomen.—The liver weighed 750 Gm., the spleen 50 Gm. The left kidney weighed 165 Gm., the right kidney 110 Gm. The adrenals were small and generally atrophic.

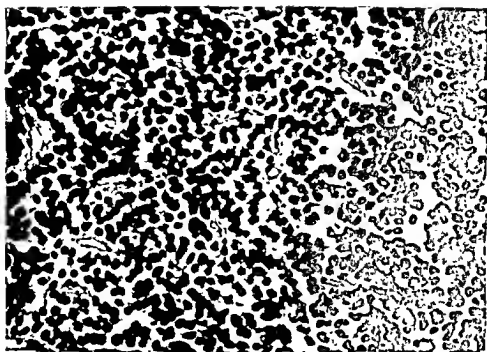


Fig. 3.—Photomicrograph of tumor (X368) showing typical arrangement of chromophobe cells; a scanty fibrous tissue stroma is seen (H. & E.).

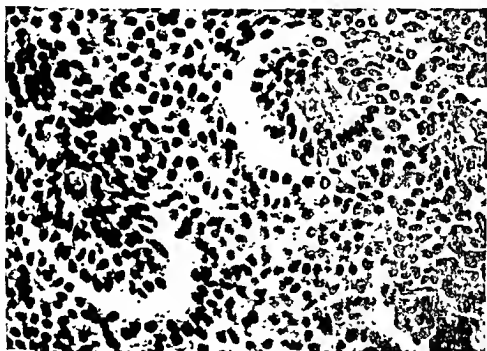


Fig. 4.—Photomicrograph of tumor (X368) showing perithelial rosettes (H. & E.).

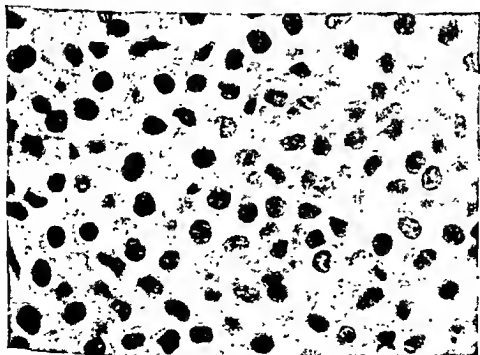


Fig. 5.—Photomicrograph of tumor (X736) showing two types of chromophobe nuclei (H. & E.).

Microscopic examination of thoracic and abdominal organs revealed nothing of interest.

Pathological Diagnoses.—Cystic chromophobe adenoma of pituitary body, Simmonds' disease, emaciation, microsplanchnia.

COMMENT

Although certain phases of the clinical study in this case are incomplete, the entire picture conforms with that of Simmonds' disease.

Admittedly many of the clinical findings might be explained on the basis of an enlarging intracranial tumor in the region of the optic chiasm with pressure on neighboring structures. Nevertheless, the clinical presence of anorexia, progressive emaciation, mental impairment, asthenia, and somnolence, combined with the post-mortem findings of pituitary destruction and microsplanchnia, justify, in our opinion, the inclusion of this case in the Simmonds' group.

The neurologic symptoms are interesting, and could be due either to "neighborhood signs" of a tumor or to anterior pituitary destruction.

From a morphologic standpoint the tumor is benign. The fact that the tumor extended into the cavernous sinus is not in contradiction to this belief. There was compression of brain substance at other points but never replacement.

The finding of pituitary tumor and Simmonds' disease in a patient in a mental and home department re-emphasizes the well-known fact that a large percentage of organic pituitary disorders are to be found in such institutions.

CONCLUSIONS

1. A case of Simmonds' disease with pituitary tumor is reported with autopsy findings.

2. The tumor, a semicystic chromophobe adenoma, had resulted in complete destruction of the pituitary body.

REFERENCES

1. Simmonds, M.: Ueber Hypophysisschwund mit tödlichen Ausgang, *Deutsche med. Wchnschr.* 40: 322, 1914.
2. Farquharson, R. F., Belt, T. H., and Duff, G. L.: Simmonds' Disease: Clinical and Pathological Observations on Four Cases, *Tr. Am. Climat. & Clin. A.* 54: 106, 1938.
3. Calder, R. M.: Anterior Pituitary Insufficiency (Simmonds' Disease), *Bull. Johns Hopkins Hosp.* 50: 87, 1932.
4. Silver, S.: Simmonds' Disease (Cachexia Hypophyseopriva), Report of a Case With Post Mortem Observations and a Review of the Literature, *Arch. Int. Med.* 51: 175, 1933.
5. Horrax, G.: *The Pituitary Gland*, Baltimore, 1938, Williams & Wilkins Co., chapter 39, page 665.

THE HISTOPATHOLOGIC CHANGES IN MYELINATED NERVE FIBERS OBSERVED BY THE POLARIZED LIGHT METHOD FOLLOWING ARTIFICIALLY INDUCED HYPERPYREXIA*

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INTRODUCTION

THE introduction of artificially induced hyperpyrexia as a modern clinical practice has aroused in the minds of many investigators the desire to know the possible histopathologic effects of varying lengths of exposure to carefully controlled body temperatures. In cooperation with the Departments of Medicine, Pathology, and Anatomy of The Ohio State University, an extensive survey of the effects of hyperpyrexia was undertaken. The scope of this communication will be limited to that portion of the work concerned with the possible histopathologic alterations of peripheral myelinated nerve fibers from animals subjected to artificially induced hyperpyrexia.

MATERIALS AND METHODS

Healthy, young adult rabbits of both sexes were chosen as experimental animals.

Hyperpyrexia-Producing Techniques.—Two distinctly different types of hyperpyrexia-producing machines were used in this investigation, viz., (1) the Kettering hypertherm, and (2) a radiotherm.

The Kettering hypertherm employed was the same machine that is used for the treatment of patients in the Outpatient Clinic of The Ohio State University Hospital. Elevation of the body temperature occurs in this machine (1) whenever the temperature of the enclosed air exceeds that of the body, and (2) whenever the humidity of this same air becomes sufficiently great to prevent evaporation of sweat from the surface of the body. It should be emphasized here that this method of producing hyperpyrexia does not depend primarily upon increased heat production by the body, but instead it elevates the body temperature by depressing the efficiency of the mechanisms for heat loss.

The radiotherm employed was designed and built by Professors E. E. Dreese and J. F. Byrne, of the Department of Electrical Engineering of the Ohio State University, for use in the study of experimental animals. The machine consists of a cabinet on two sides of which are located the electrode plates. Numerous apertures in the cabinet provide adequate circulation, and fiber-board guards prevent the animal from being burned by the electrodes. The machine produces wave lengths of 25 meters at 12,000,000 oscillations per second, and its radio

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frequency can be varied from 0.02 to 0.5 amperes, or 1 to 4 thermoamperes. Production of hyperpyrexia by this method, in contrast to hyperthermy, is due to heat generated in the animal's tissues by the passage of an electric current.

Parallel periods of hyperpyrexia were induced by the hypertherm and radiotherm techniques, respectively, and the tissue changes were compared. The investigation included the production of high temperatures for short lengths of time, as well as relatively low temperatures maintained over long intervals. Some of the animals were subjected to hyperpyrexia until death resulted, whereas others were allowed to recover. In a few instances rabbits were subjected to intermittent periods of sublethal hyperpyrexia over considerable intervals of time.

During each extended period of hyperpyrexia all animals were given approximately 50 c.c. of 1 per cent glucose in normal saline by stomach catheter at three-hour intervals. The quantity of fluids administered and the frequency of the dose were varied to that necessary to maintain a constant body weight.

Rectal temperatures in degrees Fahrenheit were recorded from each animal at fifteen-minute intervals during all periods of experimentally induced hyperpyrexia.

All animals not subjected to lethal temperatures were canceled by air emboli. Complete autopsies were performed immediately after death in all cases.

Histopathologic Method.—Preliminary observations demonstrated the necessity of a sensitive technique for the study of the possible alterations in the peripheral myelinated nerve fibers of the animals used in this experiment. The work of Baldi,¹ Setterfield and Sutton^{2, 3} Sutton, Setterfield, and Krauss,⁴ Setterfield and Baird,⁵ Setterfield and Weaver,⁶ Toomey and Weaver,^{7, 8} and Weaver and Kitchin⁹ has convinced me that the polarized light method is the most sensitive and accurate technique available for studying degenerative phenomena in peripheral myelinated nerve fibers.

The nerves to be studied were removed from the animal at autopsy, laid out without stretching on a segment of a tongue depressor, and fixed for twenty-four hours or longer in a solution of 10 per cent neutral-formalin. The nerves were then cut longitudinally on a freezing microtome at a thickness of 10 microns. The sections were floated onto slides from water and mounted in neutral glycerin.

Examination of peripheral myelinated nerve fibers by the polarized light method reveals the presence of two optically different materials, namely, (1) those exhibiting the phenomenon of birefringence, and (2) those characterized by the state of isotropism. In rotating the stage of the microscope through 360 degrees, the birefringent material can be seen to become alternately light and dark four times in each revolution. The isotropic material, on the other hand, remains dark at all points in the revolution of the stage.

In general, the appearance of peripheral myelinated nerve fibers, as seen in polarized light, closely resembles that revealed by the classic techniques. The axis cylinder, neurokeratin framework, and myelin sheath require special description.

The axis cylinder (Figs. 1 and 2), as commonly referred to in the literature, is composed of two portions, i.e., (1) an inner cylindrical rod, the true axis cylinder; and (2) an outer enveloping sheath, the non-neurofibrillated portion of the axis cylinder. In polarized light the true axis cylinder is isotropic, deep black, homogeneous, and of uniform caliber throughout its length. The non-neurofibrillated portion of the axis cylinder, as seen in polarized light, is isotropic, but less intensely black than the true axis cylinder, and very slightly

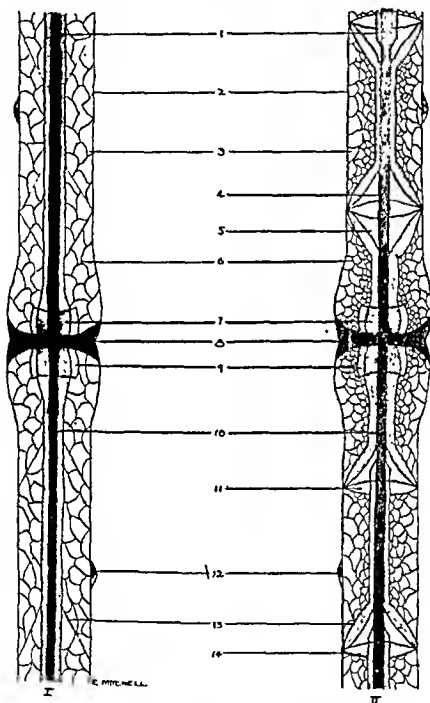


Fig. 1—Drawing of normal nerve fiber as seen in polarized light. Approximately $\times 3,000$ diameters.

1. Non-neurofibrillated portion of the axis cylinder.
2. Sheath of Schwann.
3. True myelin.
4. Naked axis cylinder.
5. Infundibulum.
6. Neurokeratin trabecula.
7. Swollen part of non-neurofibrillated portion of axis cylinder.

8. Cementing disk.
9. Bracket of Nageotte.
10. True axis cylinder.
11. Ostium of infundibulum.
12. Nucleus of Schwann cell.
13. Incisure of Schmidt-Lantermann.
14. Infundibular ring.

granular. Most authors have described the axis cylinder to be of constant caliber, except for a constriction at the level of the cementing disk, above and below which there is an enlargement. By reference to Fig. 1 it can be seen that it is the non-neurofibrillated portion of the axis cylinder only that is involved in this enlargement and in the constriction.

EXPERIMENTAL PROCEDURE, OBSERVATIONS AND RESULTS

GROUP	NO. OF ANIMALS	FEVER MACHINE	TREATMENT	MICROSCOPIC APPEARANCE OF NERVE FIBERS
A	16		Control	Normal (Figs. 1 and 2). The nerve fibers in this species of animal are somewhat peculiar in that the non-neurofibrillated portion of the axis cylinders show some slight peripheral irregularity, and are characterized by a more or less pronounced vacuolization just above and below the cementing disk. Whenever these terms are employed in the description of nerves from experimental animals, it is to be understood that they represent changes over and above that seen in a normal fiber.
B	4	Radiotherm	Lethal temperature	Normal
C	2	Hypertherm	Lethal temperature	Normal
D	2	Radiotherm	109.0° F. until death	Extensive peripheral irregularity and marked vacuolization at the nodes of Ranvier of the non-neurofibrillated portion of the axis cylinders. Numerous petechiae in the nerve trunk. In one animal degeneration was more marked in 60 per cent of the fibers. In the latter (Fig. 4) the myelin sheath contained much isotropic material; approximately 25 per cent of the true axis cylinders were fragmented; the non-neurofibrillated portions of the axis cylinders were badly swollen fragmented, and more markedly vacuolated; the neurokeratin framework was swollen and isotropic; nodes of Ranvier were hardly recognizable; and the sheath of Schwann apparently contained more nuclei than normal.
E	2	Hypertherm	109.0° F. until death	The nerve fibers showed some edema; the axis cylinders, both the true and non-neurofibrillated portions, showed an extreme degree of vacuolization and fragmentation in the majority of the fibers; the myelin sheath contained numerous globules of isotropic material; the nodes of Ranvier were scarcely recognizable, but when seen were swollen and filled with isotropic material; the neurokeratin framework was either isotropic or hidden in the adjacent degenerated debris; the sheath of Schwann was edematous (Fig. 3).
F	2	Radiotherm	108.0° F. until death. One animal canceled after 13 hours	All fibers showed peripheral irregularity of the non-neurofibrillated portion of the axis cylinders. In half the animals the neurokeratin framework was swollen and isotropic, and there was edema of the non-neurofibrillated portion of the axis cylinders. One animal showed vacuolization and fragmentation of the non-neurofibrillated portion of the axis cylinders, and fragmentation of a large proportion of the true axis cylinders, together with considerable derangement of the nodes of Ranvier. Two animals showed isotropic material in the myelin sheaths.

EXPERIMENTAL PROCEDURE, OBSERVATIONS, AND RESULTS—CONT'D

GROUP	NO. OF ANIMALS	FEVER MACHINE	TREATMENT	MICROSCOPIC APPEARANCE OF NERVE FIBERS
G	2	Hypertherm	108.0° F. until death	The myelin sheaths contained some isotropic material; the neurokeratin framework was swollen and isotropic; in about 50 per cent of the fibers the non-neurofibrillated portions of the axis cylinders showed peripheral irregularity and were markedly vacuolated, and some showed considerable edema.
H	2	Radiotherm	107.0° F. until death	Normal
I	2	Hypertherm	107.0° F. until death	The non-neurofibrillated portion of the axis cylinders showed some edema, peripheral irregularity, and marked vacuolization. In one animal the neurokeratin framework was isotropic and swollen slightly, and one petechia was observed.
J	1	Hypertherm	103.5° F. for 43½ hours and canceled	The neurokeratin framework was slightly swollen and 10 per cent of the fibers showed edema of the non-neurofibrillated portion of the axis cylinders.
K	2	Radiotherm	Intermittent periods of 106.5° F. Total of 18 hours. Seven days recovery	In one animal there was a slight swelling, peripheral irregularity, and vacuolization of the non-neurofibrillated portion of 15 per cent of the axis cylinders. In the other animal these changes were more marked, especially at the nodes of Ranvier. Ten per cent of the fibers in this animal showed fragmentation of the true axis cylinders and isotropic material in the myelin sheaths.
L	2	Hypertherm	Intermittent periods of 106.5° F. Total of 18 hours. Seven days recovery	One animal showed slight vacuolization of the non-neurofibrillated portion of the axis cylinders. In the other animal the fibers were normal, except for those in one small fasciculus which were characterized by a marked vacuolization of the non-neurofibrillated portion of the axis cylinders.
M	2	Hypertherm	Intermittent periods of 108.0° F. Total of 50 hours. Seven days recovery	One animal showed peripheral irregularity of the non-neurofibrillated portion of the axis cylinders and marked vacuolization in 10 per cent of these structures. In the other animal the non-neurofibrillated portion of the axis cylinders was slightly vacuolated, and the neurokeratin framework was slightly swollen and isotropic.
N	2	Radiotherm	108.0° F. for 8 hours. Thirty days recovery	In one animal the nerves were normal. In the other animal the fibers showed a marked vacuolization of the entire length of about 10 per cent of the non-neurofibrillated portions of the axis cylinders, and some isotropic material in the myelin sheaths.
O	2	Hypertherm	108.0° F. for 8 hours. Thirty days recovery	The myelin sheaths contained numerous globules of isotropic material; the neurokeratin framework was swollen and isotropic; there was peripheral irregularity of the non-neurofibrillated portion of the axis cylinders in 50 per cent of the fibers, and marked vacuolization of the entire length of these structures in about 25 per cent of the fibers. In one animal there was considerable derangement of the nodes of Ranvier.

In a normal nerve fiber the neurokeratin framework (Fig. 1) is very difficult to see because its index of refraction is only slightly different from that of the myelin sheath. Under an oil-immersion objective the neurokeratin framework appears as a thin, isotropic, skeletal system of anastomosing trabeculae, which bridge across the myelin sheath from the non-neurofibrillated portion of the axis cylinder to the sheath of Schwann. This spongy neurokeratin framework contains potential cavities of varying size—potential because they are filled with true myelin. In certain edematous and degenerative changes of the nerve fiber the neurokeratin trabeculae become larger and somewhat darker.



Fig. 2.—Rabbit 100, Group A. $\times 1,575$ diameters.

The myelin "sheath" (Fig. 1) is, in polarized light, the only structure in the nerve fiber exhibiting the phenomenon of birefringence. Under high magnification it becomes apparent that the myelin is not in the form of a sheath, but instead is present as variously-shaped globules confined to the interstices of the neurokeratin framework. As degenerative changes in a nerve fiber progress, the myelin progressively loses its birefringency and becomes isotropic.

DISCUSSION

An examination of the histopathologic changes noted in the peripheral nerves of the animals presented in this study reveals the necessity of a declaration of their reversibility or irreversibility.

During earlier studies on poliomyelitis (Toomey and Weaver⁷) it was pointed out that fragmentation of the true axis cylinders was always associated with some degree of muscular dysfunction. During acute stages of polio-

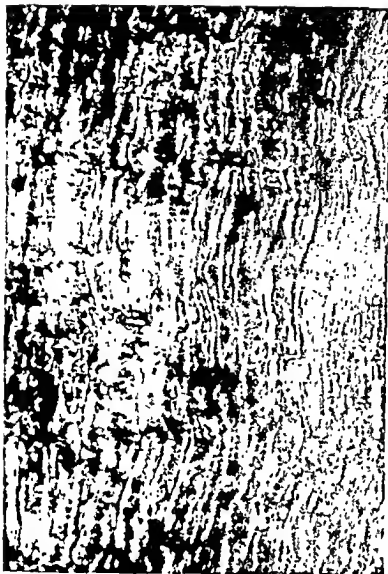


Fig. 3.—Rabbit 146, Group E. $\times 1,575$ diameters.



Fig. 4.—Rabbit 150, Group D. $\times 1,575$ diameters.

myelitis the number of true axis cylinders showing fragmentation were noted. If animals under similar conditions were allowed sufficient time to recover and their nerves again examined, the number of nerve fibers showing complete degeneration corresponded closely to the number of nerve fibers showing fragmentation of the true axis cylinders during the acute stages of the disease. From this observation it was inferred that fragmentation of the true axis cylinder is irreversible in character, though it must be kept in mind that regeneration of the fiber may occur if the cell body is not destroyed.

Peripheral irregularity, vacuolization, and fragmentation of the non-neurofibrillated portion of the axis cylinder may be considered reversible in character, although these changes are indicative of injury to the nerve fiber. The intensity of the injury probably becomes progressively greater in the order that they have been named. The truth of this statement is indicated by the fact that in acute stages of poliomyelitis almost every nerve fiber is affected by these changes. If an animal showing these changes (for example, an animal suffering from acute poliomyelitis but showing little muscular dysfunction) is allowed sufficient time to recover and their nerves are again examined, essentially all the nerve fibers will appear normal.

It should be pointed out that some vacuolization, especially at the nodes of Ranvier, and some peripheral irregularity throughout its entire length, is characteristic of the non-neurofibrillated portion of the axis cylinders of fibers from a normal animal. Whenever these terms were employed in the description of the nerves from the experimental animals employed in this investigation, it is to be understood that they represent a change over and above that seen in a normal fiber.

The presence of isotropic granules in the myelin sheaths is undoubtedly an example of an irreversible type of degenerative change, although the presence of these granules is not necessarily indicative of neurologic dysfunction. From a series of isolated observations on the nerves of animals subjected to varying types of stress, during both acute and recovery stages, it is believed that these isotropic granules are removed by the phagocytic activity of the neurilemma cells.

Edema of the fiber as a whole, or of any of its parts, with the single possible exception of the true axis cylinder; irregularity and waviness of the fiber; and swelling with increased isotropism of the neurokeratin framework, are all considered to be reversible in character, and, when present alone, to be indicative of rather mild injury to the nerve fiber.

Although it was in most cases considerably more difficult to maintain a constant temperature by radiothermy than by hyperthermy, a cursory examination of the descriptions of the peripheral nerves reveals that degenerative changes were more marked in the series subjected to radiothermy. There appear to be only two possible explanations for this seeming dissimilarity: (1) The difference in the microscopic picture of the nerves of the animals in the two series subjected to practically identical periods of average temperature may, in some cases at least, be explained by the fact that animals in the radiothermy group were allowed to move about freely, whereas the production of hyperpyrexia by hyperthermy necessitated restraining the animals so that their heads could be

retained outside the machine. Examination of the gross necropsy findings in the animals in both the radiotherapy and hyperthermy groups revealed a marked derangement of the blood vascular system. Since the efficiency of peripheral circulation is influenced greatly by the tone and activity of limb muscles, it is only logical to assume that restraining the animal from moving would tend to depress the efficiency of the already nearly (or actually) inadequate circulatory system and, thereby, tend to hasten anoxia in the extremities. Since it is well known that nervous tissue as a whole is particularly sensitive to this condition, it might be utilized as an explanation, in part at least, for the more pronounced picture of degeneration in the nerves of the hyperthermy animals. (2) The passage of short radio waves through the tissues of the animal may in some manner unknown to me serve to counteract the deleterious effects of hyperpyrexia or of its sequelae.

Experiments are now in progress in which anoxia has been produced without elevation of the body temperature. Comparison of the tissues of these animals with those just described will, we trust, enable us to make a more definite statement as to the etiology of the histopathologic changes following artificially induced hyperpyrexia by the methods of radiotherapy and hyperthermy.

The description of the histopathologic changes noted in the peripheral nerves of the different groups of animals reported in this paper has been summarized. No further discussion is indicated.

SUMMARY AND CONCLUSIONS

In cooperation with the Departments of Medicine, Pathology, and Anatomy of The Ohio State University, an extensive program was undertaken, the primary object of which was to determine the possible histopathologic effects of artificially induced hyperpyrexia by the methods of hyperthermy and radiotherapy. The present paper has been confined to the changes observed in the peripheral myelinated nerves of the animals used in this joint investigation.

Using rabbits as the experimental animal, the investigation extended from the production of high temperatures for short lengths of time to relatively low temperatures over long intervals. Some of the animals were subjected to hyperpyrexia until death resulted, whereas others were allowed to recover. Finally, a few rabbits were subjected to intermittent periods of hyperpyrexia over considerable intervals of time.

From examination of the peripheral nerves by the polarized light method I have reached following conclusions:

1. Degenerative changes were more marked in the series subjected to hyperpyrexia by hyperthermy than in the series subjected to comparable degrees by radiotherapy. A possible reason for this was discussed.
2. There was no change from the normal in the nerves of rabbits subjected to hyperpyrexia by hyperthermy or radiotherapy, when the duration of hyperpyrexia fell between thirty-three and seventy-five minutes, and the terminal temperatures varied from 111.5° F. to 111.9° F.
3. Hyperpyrexia by either method, in which the temperature varied between 108.7° F. and 109.1° F. , and the duration of hyperpyrexia varied from four

hours and five minutes to seven hours and two minutes, resulted in marked degenerative changes of irreversible and reversible types in the peripheral nerves of rabbits.

4. In rabbits subjected to hyperpyrexia by radiotherapy or hyperthermy until death, and in which the temperature did not exceed 108.3° F., the peripheral nerves were characterized by degenerative changes of the reversible type and, furthermore, the intensity of these changes was directly proportional to the height of the temperature. It is quite possible that changes of this nature are responsible for the occasional cases of neuritis seen in patients following artificial fever therapy.

5. In rabbits subjected to intermittent periods of hyperpyrexia (approximately one exposure of from three to six hours' duration each week, and varying from 106.1° F. to 107.84° F.)—duration of hyperpyrexia extending from eighteen to fifty hours, with one week recovery time allowed between last exposure and time of cancellation, the peripheral nerves showed only mild intensities of the reversible types of degeneration.

6. In rabbits subjected to hyperpyrexia by hyperthermy or radiotherapy in which the temperature varied between 107.65° F. and 108.05° F. for eight hours, and in which thirty days intervened between the time of exposure and the time of cancellation, the peripheral nerves showed less change in the radiotherapy group than in the hyperthermy group. In both groups, however, there was evidence of a rapid return to the normal.

I wish to express my sincere appreciation to Dr. Linden F. Edwards, associate professor of anatomy, Ohio State University, for his critical reading of and help in arranging this report; to Dr. Julien M. Goodman, then a medical student, and to Dr. Milton Berman, then a dental student, for the many hours they so patiently and skillfully devoted to the experimental procedure involved in this investigation; to Dr. Charles A. Doan, professor of medicine, Ohio State University, for his help in arranging the details of the experimental procedure of this investigation, for furnishing the experimental rabbits, and for his generosity which made available the facilities and equipment of his department; to Dr. Paul C. Kitchin, associate professor of Dentistry, for his permission to use the polarized light microscope and the photographic equipment of the College of Dentistry; and to Mrs. Elizabeth Arrowsmith for her faithful execution of the drawing presented in this paper.

REFERENCES

1. Baldi, F.: *Uteriori ricerche a luce polarizzata sulla degenerazione Walleriana*, Riv. de pat. nerv. 35: 158, 1930.
2. Setterfield, H. E., and Sutton, T. S.: The Use of Polarized Light in the Study of Myelin Degeneration. I. The Appearance and Progress of Degeneration After Transection of the Sciatic Nerve of the White Rat, *Anat. Rec.* 61: 397, 1935.
3. Setterfield, H. E., and Sutton, T. S.: The Use of Polarized Light in the Study of Myelin Degeneration. II. The Degeneration of Myelinated Nerves in Avitaminosis A in the White Rat, *J. Nutrition* 9: 645, 1935.
4. Sutton, T. S., Setterfield, H. E., and Krauss, W. E.: Nerve Degeneration Associated With Avitaminosis A in the White Rat, *Bulletin No. 545, The Ohio Agricultural Experimental Station, Wooster, Ohio*, 1934.
5. Setterfield, H. E., and Baird, T. T.: Polarized Light Technique. A Comparison With the Marchi Method, *Stain Technol.* 11: 41, 1936.
6. Setterfield, H. E., and Weaver, H. M.: Some Cytological Observations on Peripheral Myelinated Nerve Fibers as Observed by the Polarized Light Method, *Ohio J. Sc.* 37: 65, 1937.
7. Toomey, J. A., and Weaver, H. M.: Poliomyelitis Virus and the Degeneration of Peripheral Nerves, *Am. J. Dis. Child.* 53: 79, 1937.
8. Toomey, J. A., and Weaver, H. M.: Experimental Paralysis in Monkeys Completely Recovered From Poliomyelitis Produced by Cultures of Material From Patients With Measles, *Am. J. Dis. Child.* 57: 541, 1939.
9. Weaver, H. M., and Kitchin, P. C.: Observations on Peripheral Nerves Following Local Injections of: (1) Isotonic Solutions of Varying pH and (2) "Iso-pH" Solutions of Varying Tonicity. To be published. *J. Dent. Research.*

CLINICAL CHEMISTRY

EFFECT ON THE NEWBORN OF VITAMIN K ADMINISTERED TO MOTHERS IN LABOR*

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IN AN effort to eliminate hemorrhagic disease of the newborn, we have given synthetic vitamin K, in gelatin capsules containing 1 mg. of 2-methyl-1,4-naphthoquinone in corn oil,[†] to a series of 100 women in labor. The prothrombin clotting time was then determined on the babies, starting the first or second day of life, and usually repeated every other day until at least four determinations had been made. In some few cases babies were taken on successive days, when checks seemed desirable, and others on occasion skipped two days. Each determination reported was the result of three or more successive readings. A similar study was made on a series of 100 controls.

For such a study a method economical in blood was necessary. An adaptation of the bedside method of Quick,¹ which gives a practical measure of the tendency to bleed, proved satisfactory. A total of 20 c.mm. of blood, drawn from a heel puncture, was discharged directly from the pipette into a similar amount of thromboplastin on a depression slide, and the mixture was stirred with a needle. The needle was then drawn through the blood until a clot was demonstrated. The interval between the contact of the blood with the thromboplastin and the first clot, read on a stop watch, is the prothrombin clotting time. Free flowing blood was necessary, the puncture being wiped clean each time before filling the pipette. Squeezing or milking tended to alter the results, and repeated tests from the same puncture tended to give faster clotting times. Duplicates could usually be obtained agreeing to within 0.5 second. When they did not agree, there was generally a recognizable reason, in which case the value obtained under the more satisfactory conditions was accepted.

The thromboplastin was prepared from rabbit brain as described by Quick,² and the dried product, well stoppered, was kept satisfactorily in the refrigerator. We found it advisable, however, to prepare fresh saline suspensions every third day, as with the frequent use necessary, we found a tendency toward slower results after the fourth day. The suspension was prepared by adding 0.3 Gm. of the dehydrated rabbit brain to 5 c.c. of normal saline containing 0.1 c.c. sodium oxalate (1.34 Gm. per 100 c.c.) and incubating for fifteen minutes in a water bath at 50° C. The stoppered suspension was then centrifuged very slowly for three minutes, and the milky liquid was decanted off for use. This was kept tightly stoppered in the refrigerator when not in use.

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[†]Thyloquinone or Microcaps were supplied by E. R. Squibb & Sons, New York.

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We did not find the device of using the clotting time of a normal adult as a standard satisfactory for determining the percentage of prothrombin in the sample under investigation.³ Repeated tests on normal adults, using the same procedure with finger blood substituted for the heel puncture, showed a considerable variation from time to time. In one person, H.S., tested on 14 different occasions, there was an extreme variation of from 14 seconds to 23.7 seconds. Another, J. M., varied from 15.5 seconds to 24.2 seconds, and a third, V. M., from 16.8 seconds to 24.2 seconds. Furthermore, there is a difference between different individuals taken at the same time under the same conditions. On March 2, 1940, this amounted to almost four seconds between four normal adults; on November 26, 1940, to 1.6 seconds between two adults; and on December 19, 1940, to almost one second between two others. For this reason, we consider it better to use the average time, 19.2 seconds, determined from 21 different individual adults, as shown in Table I. We also feel it more accurate to report all findings directly in time, rather than converting them by formulas to percentages of prothrombin.

TABLE I
DETERMINATION OF PROTHROMBIN CLOTTING TIME ON NORMAL ADULTS
Modified Bedside Method
(Time in seconds. Thromboplastin preparation one to four days old.)

DETERMINATIONS MADE	PATIENT	NO. TESTS	AVERAGE TIME
3/ 2/40 to 9/ 6/40	H. S.	14	19.3
3/ 2/40 to 12/19/40	J. M.	7	20.3
3/ 2/40 to 4/23/40	V. M.	4	19.8
3/ 2/40	R. M.	1	20.9
5/ 1/40 to 5/ 2/40	F. C.	2	21.1
8/ 6/40	S. L.	1	14.9
8/ 6/40	M. G.	1	15.2
8/14/40	M. K.	1	15.6
9/20/40 to 9/21/40	D. D.	2	19.4
9/23/40 to 9/24/40	E. S.	2	20.2
10/ 7/40	J. S.	1	19.4
10/14/40	D. B.	1	18.6
10/30/40	H. H.	1	21.2
10/31/40	M. B.	1	21.7
11/ 5/40	M. S.	1	19.2
11/20/40	B. S.	1	19.5
11/26/40	M. P.	1	19.4
11/26/40	M. M.	1	17.8
11/29/40	A. H.	1	21.6
12/19/40	J. C.	1	18.7
12/19/40	G. C.	1	19.6
Average time for all patients			19.2

A summary of our results is presented in Table II. It is evident that the series receiving the vitamin K shows a much more rapid clotting time than the controls. The averages for each of the ten days studied is faster, the maximum values are lower in every group, and even the minimum findings are lower than those of the controls until the ninth and tenth days, by which time all infants are supposed to have re-established normal clotting times.⁴⁻⁶ More striking yet, however, is the fact that on the first and second days of life nearly a third of all the controls done were higher than the maximum found in the experimental series for those same days. On the third and fourth days over half of the controls were over the vitamin K series maximum, and even as late as

the sixth day a third showed a slower clotting time than the slowest treated patient. After the seventh day recovery is apparent, since both series approach the normal adult time. It is worthy of note that the maximum figures given in the table are not successive determinations on one or two babies, but in the ten days, are from 7 different persons.

We tried to give the vitamin K capsules with water during the first stage of labor from four to ten hours before delivery, and before any anesthesia. Naturally this was not always possible, and in 11 persons, where it was evident that the time would be longer, a second capsule was given. In 18 other women delivery followed in less than four hours. Of these, 2 showed about the average clotting time, 9 were faster than the average, and 7 were slower; none of the 7, however, was as slow as the average for the control series. There were also 18 patients who were not given a second capsule, although delivery came after ten hours. Of these, 8 were slower than the average for the treated series, 4 were faster, 5 were above average for the first day or two of life, then fell below the average, and one started slow then became better than average. One of these, below average throughout, by the fifth day had fallen below the untreated average. From this we conclude that normally absorption of the drug is fairly rapid, four hours apparently being more than ample, but that the maximum effect begins to fade after about ten hours. The 5 babies that started faster than normal only to fall below later would indicate they had more of a supply of the vitamin in their systems at birth than untreated babies, but not enough to last until they had established their own supply.

One case in the treated series stood out as a marked exception to all the rest. This mother was given two capsules, the first eleven hours forty-five minutes and the second four hours before delivery. Delivery was by breech extraction, with a large but normal baby. The five prothrombin clotting time determinations are given in Table II, but because they are so markedly different from the entire series they are not included in the averages or maximum values. Even so this one exception does not approach the higher figures of the untreated cases, as there were 9 untreated babies higher on the second day, 2 on the third, and 2 on the sixth. We have no explanation for this peculiar case, but suppose it must have been due to failure of the mother to absorb the vitamin. No jaundice was apparent in either mother or baby, and no determinations were made on the mother.

No clinical signs of bleeding were observed in either series. A few jaundiced babies were noted, usually about the fourth or fifth day, but these invariably had rapid prothrombin times, treated or untreated. None of the babies were exposed to any strain on their systems, and it may well be that, as suggested by Quick,⁷ they were delicately balanced, and clinical bleeding would have followed any disturbing factor.

We have observed a few cases where infants with prolonged bleeding or clotting times, or with clinical evidence of bleeding, have been treated directly. In these instances we have given 1 mg. doses, in oil, dropping the solution on the back of the tongue from a dropper or small syringe, without a needle. At first as much as 5 mg. in twenty-four hours was given; later this was reduced to 1 mg. every eight hours for twenty-four hours. In every instance the treatment has

TABLE II
SUMMARY OF POSTHUMOUS TITRATIONS FOR VITAMIN K PREPARATION
Modified Redside Method

(Time in seconds. Data covering a study of 100 babies from whom given vitamin K during labor and 100 controls from untreated mothers.)

	1ST DAY		2ND DAY		3RD DAY		4TH DAY		5TH DAY		6TH DAY		7TH DAY		8TH DAY		9TH DAY		10TH DAY	
	No.	Avg.	No.	Avg.	No.	Avg.	No.	Avg.	No.	Avg.	No.	Avg.	No.	Avg.	No.	Avg.	No.	Avg.	No.	Avg.
Daily determinations																				
Treated	10	21.7	89	20.9	41	20.7	55	21.2	59	20.7	78	20.4	11	19.9	54	17.9	17	20.1	3	19.1
Controls	19	25.1	80	24.4	29	23.1	62	23.3	51	20.7	53	20.7	21	21.7	32	22.5	11	21.5	5	21.4
Maximum																				
Treated		27.7		22.3		28.5		20.0		26.8		29.0		20.1		27.4		28.1		24.6
Controls		29.6		20.2		114.0		24.6		29.7		29.2		24.7		15.6		24.9		29.8
Minimum																				
Treated		17.2		15.9		16.9		15.5		15.2		15.3		14.1		11.1		17.2		16.9
Controls		17.8		12.1		19.9		17.7		19.1		16.9		17.1		13.8		15.9		15.2
Exception*																				
Treated		12.9		59.0							17	17.5				10.3	1			32.3
No. of controls over maximum vitamin K values	6		25		13		31		5				1		9		1		1	

*Only high value found in the entire treated series, not included in the averages.

been satisfactory, all clinical signs clearing up within twenty-four hours, and the prothrombin time returning to about the normal average. In one such case a prothrombin time of over ten minutes fell to twenty-five seconds within forty-eight hours after treatment started.

SUMMARY AND CONCLUSIONS

The average prothrombin clotting time of babies whose mothers were given synthetic vitamin K (2-methyl-1,4-naphthoquinone) by mouth during labor was much faster than the average of a similar untreated series, and close to the normal adult average.

From 15 to 50 per cent of the control series determinations made during the first six days were higher than the highest value in the treated series for the corresponding day.

One milligram of the vitamin in corn oil during the first stage of labor, four to ten hours before delivery, proved effective.

Three milligrams in twenty-four hours at eight-hour intervals, given direct to the baby by mouth, has been sufficient to control bleeding and reduce prolonged prothrombin clotting times to normal.

Administration of the synthetic vitamin K to women in labor has been adopted as a routine procedure at the Maternity Hospital and the Cleveland City Hospital.

REFERENCES

1. Quick, A. J.: Determination of Prothrombin, *Proc. Soc. Exper. Biol. & Med.* 42: 788, 1939.
2. Quick, A. J.: The Nature of the Bleeding in Jaundice, *J. A. M. A.* 110: 1658, 1938.
3. Smith, H. P., Ziffren, S. E., Owen, C. A., and Hoffman, G. R.: Clinical and Experimental Studies on Vitamin K, *J. A. M. A.* 113: 380, 1939.
4. Owen, C. A., Hoffman, G. R., Ziffren, S. E., and Smith, H. P.: Blood Coagulation During Infancy, *Proc. Soc. Exper. Biol. & Med.* 41: 181, 1939.
5. Quick, A. J., and Grossman, A. M.: Prothrombin Concentration in Newborn, *Proc. Soc. Exper. Biol. & Med.* 41: 227, 1939.
6. Kato, K., and Poncher, H. G.: The Prothrombin in the Blood of Newborn Mature and Immature Infants, as Determined by the Micro Prothrombin Test, *J. A. M. A.* 114: 749, 1940.
7. Quick, A. J., and Grossman, A. M.: Nature of Hemorrhagic Disease of the Newborn: Delayed Restoration of Prothrombin Level, *Am. J. M. Sc.* 199: 1, 1940.

BLOOD CHEMISTRY OBSERVATIONS IN LEUCEMIAS*

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THE object of this paper is to report the blood chemistry findings on 9 patients with myelogenous leucemia, and 4 patients with lymphatic leucemia. Acute, chronic, and aplastic phases of the disease are represented.

It has been observed for years that when the blood of patients with leucemia is drawn from a vein and allowed to stand for a short time a white fatty layer, or a fatty turbidity develops. This seemed to bear no relationship to food taking, and the sera drawn twelve hours after a meal were often as turbid as those drawn after the patient had had breakfast. When the blood of patients with leucemia was spread on a slide for staining, it had a peculiar granular appearance not met with in any other type of blood dyscrasia. These observations led us to believe that in this disease there might be some dysfunction of the fat metabolism.

In 1925 Backman, Daland, and Weld reported some observations on the phosphorus content of whole blood, plasma, and cells. They concluded that in myelogenous leucemia there was an increase of the phosphorus in the whole blood due to the increased phosphorus in the cells and that the serum phosphorus remained the same. They also concluded that the total phosphorus content of the cells depended on the number of immature cells present. We felt that in addition to the increased phosphorus and phospholipids in the cells that there must also be an increase in the lipoids of the blood plasma and serum in order to produce the turbidity noted in oxalated blood and the granular appearance seen when making slides.

Due to the difficult methods for lipid determinations and the large volumes of blood required, it has been impossible to get complete determinations on all patients. We have, however, done cholesterol, cholesterol ester, lipid phosphorus, lecithin, total lipid, and fatty acid determinations. The results of the lipid determinations are listed in Table I. In addition we have done amino acid, inorganic calcium, phosphorus, and few nonprotein nitrogen determinations. Only two albumin and globulin ratios have been done.

All the determinations were done by one of us (M. R. F.) in the laboratories of the Presbyterian Hospital of Chicago. The methods used are the standard ones employed in the hospital laboratories and are listed in the references. Our results are summarized in Tables I and II. Table I dealing with lipid chemistry and Table II with the other observations that we were able to make.

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TABLE I

NO.	DIAG- NOSIS	HB.	R.B.C.	W.B.C.	BLAST (%)	TOTAL CHOLES- TEROL (MG. PER 100 C.C.)			FREE CHOLES- TEROL (MG. PER 100 C.C.)			CHOLESTEROL ESTERS (MG. PER 100 C.C.)			TOTAL LIPIDS (MG. PER 100 C.C.)			LIPOID PHOSPHORUS (MG. PER 100 C.C.)			LECITHINS (MG. PER 100 C.C.)			FATTY ACIDS (MG. PER 100 C.C.)		
						WB	P	S	WB	P	S	WB	P	S	WB	P	S	WB	P	S	WB	P	S	WB	P	S
1	L.L.	54	2.09	86.2	None	210	163	213	94	101	87	69	112	157	1.76	1.63	8.08	11.9	10.4	8.08	0.298	0.259	0.202	624	404	501
2	M.L.	76	4.45	8.85	None	224	217	253	110	133	59	107	133	1.33	1.76	1.79	9.2	13.6	10.6	9.2	0.339	0.265	0.229	722	757	583
3	M.L.	61	4.28	229.0	8	224	145	145	72	74	71	73	73	1.33	1.66	1.73	7.2	16.3	6.5	7.2	0.413	0.162	0.179	923	623	670
4	M.L.	58	3.25	400.0	6	205	133	143	72	75	140	61	68	2.01	1.33	1.56	6.4	10.1	6.3	6.4	0.253	0.156	0.159	923	364	587
5	L.L.	28	1.20	190.0	90	199	199	199	81	81	118	118	118	1.66	1.66	1.66		19.9	19.9		0.497			499		
6	M.L.	59	3.39	239.0	5	222	222	222	156	156	66	66	66	2.39	2.39	2.39		18.8	18.8		0.479					
7	M.L.	57	3.38	331.0	7	240	240	240	155	155	85	85	85	1.83	1.83	1.83		19.9	19.9		0.498					
8	M.L.	60	3.40	297.0	5	240	240	240	155	155	85	85	85	2.33	2.33	2.33		30.3	30.3		1.25					
9	M.L.	45	2.88	381.0	10	151	151	151	103	63	53	67	196	1.12	1.23	1.83		85.9	52.0	76.1	2.14	1.13	1.99			
10	M.L.	60	3.65	50.9	19	153	129	293	186	186	141	141	141	1.73	1.73	1.73		101.0	101.0		1.11					
11	M.L.	40	2.83	37.4	70	327	327	327	73.3	73.3	168	168	168	1.73	1.73	1.73		43.7	43.7		2.32					
12	M.L.	40	2.69	319.0	45	175	175	175	73.3	73.3	168	168	168	1.73	1.73	1.73		43.7	43.7		2.32					
13	M.L.	78	4.45	4.35	None	232	232	232	73.3	73.3	168	168	168	1.73	1.73	1.73		43.7	43.7		2.32					
14	L.L.	30	1.98	151.0	78	116	116	116	71	71	33	33	65	1.27	1.27	1.27		32.5	32.5		1.09		0.812			
15	L.L.	30	1.98	108.0	10	143	137	121	67	57	38	70	41	1.2	1.33	1.26		8.0	5.76	2.10	0.200	0.144	0.128			
16	L.L.	35	1.48	7.20	5	132	132	132	106	106	38	70	41	1.2	1.33	1.26		21.8	21.8		0.546		0.506			
17	L.L.	37	1.37	19.6	10	160	160	160	115	115	115	115	115	1.93	1.93	1.93		33.0	33.0		0.827					
18	M.L.	65	3.31	49.0	80	231	231	231	115	115	115	115	115	1.93	1.93	1.93		8.76	8.76		0.219					
19	M.L.	65	3.24	60.0	91	266	266	266	134	134	132	132	132	1.66	1.66	1.66		4.17	4.17		0.104					
20	M.L.	50	2.74	70.4	50	67	67	67	26	26	41	41	41	1.66	1.66	1.66		4.92	4.92		0.122					
21	M.L.	78	4.08	16.8	None	155	155	155	74	74	81	81	81	1.66	1.66	1.66		5.87	5.87		0.147					

10. A biologic assay on a three-day sample of urine of one of the myelogenous leucemia patients done through the courtesy of Dr. F. C. Koch in his laboratories showed: international units of androgen per day 15; estrogens as equivalent of theelin per day 12 gamma. The value for androgen is very low for normal males.

CONCLUSIONS

1. Total lipoids in all cases were greatly elevated, irrespective of the type of leucemia and the height of the blood count.

2. Total and esterified cholesterol determinations were normal in all but one case.

3. The inorganic calcium and phosphorus determinations were normal. Non-protein nitrogen determinations were normal.

4. Four amino acid determinations were normal, and one was elevated.

5. Fatty acids were elevated much above normal.

6. The difference between the total lipoids and the sum of the cholesterol, fatty acids, and phospholipid fractions was greater in all cases than the normally expected values for normal fats.

7. Lipoid phosphorus was elevated in only a third of the cases and did not bear a constant relationship to the height of the white count and the number of blast cells present.

Note.—Blood lipid determinations were all done by the Bloor method and were begun before the work of Christensen¹² and Van Slyke and Folch¹³ was published. This study of the lipoids in leucemias is being continued in our laboratories by both the Bloor method and the modifications suggested by the authors mentioned.

REFERENCES

1. Mathews, A. P.: *Physiological Chemistry*, Baltimore, 1939, Williams & Wilkins Co.
2. Maegeli, O.: *Blutkrankheiten und Blutdiagnostik*, ed. 5, Berlin, 1931, Julius Springer.
3. Forkner, C. E.: *Leukemia and Allied Disorders*, New York, 1938, The Macmillan Company.
4. Peters, J. P., and Van Slyke, D. D.: *Quantitative Clinical Chemistry*, Baltimore, 1931, Williams & Wilkins Co., Vol. 1.
5. Buckman, J. E., Daland, C. A., and Weld, M.: *Blood Phosphorus in Chronic Myelogenous Leukemia*, *Arch. Int. Med.* 35: 389, 1925.
6. Bloor, W. R.: *A Method for the Determination of Fat in Small Amounts in Blood*, *J. Biol. Chem.* 17: 337, 1914.
7. Bloor, W. R.: *The Determination of Small Amounts of Lipid in Blood Plasma*, *J. Biol. Chem.* 77: 53, 1928.
8. Bloor, W. R.: *Determination of Fatty Acids (and Cholesterol) in Small Amounts of Blood Plasma*, *J. Biol. Chem.* 52: 191, 1922.
9. Peters, J. P., and Van Slyke, D. D.: *Quantitative Clinical Chemistry*, Baltimore, 1932, Williams & Wilkins Co., Vol. 2, pp. 882, 884.
10. Bloor, W. R., and Knudson, A.: *The Separate Determinations of Cholesterol and Cholesterol Esters in Small Amounts of Blood*, *J. Biol. Chem.* 27: 106, 1916.
11. F. C. Koch: *Practical Methods in Biochemistry*, ed. 2, Baltimore, 1937, William Wood & Co., p. 143.
12. Christensen, H. N.: *Contaminants of Blood Phospholipids*, *J. Biol. Chem.* 129: 531, 1939.
13. Van Slyke, D. D., and Folch: *Nitrogenous Contaminants of Petroleum Ether Extracts*, *J. Biol. Chem.* 129: 539, 1939.

DETERMINATION OF BLOOD PYRUVATE IN VITAMIN B₁ DEFICIENCY*

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UNDoubtedly one of the most striking changes occurring in the tissues and body fluids in vitamin B₁ deficiency is the abnormal accumulation of carbonyl compounds, particularly pyruvic acid, as the result of incomplete or disturbed metabolism of carbohydrate, as demonstrated by Thompson and Johnson,¹ and by Johnson, Meiklejohn, Passmore, and Thompson.² Extensive investigations by Peters and his associates have led also to the hypothesis that vitamin B₁ functions as a coenzyme in the oxidative catabolism of glucose, particularly in the decarboxylation of pyruvic acid.³ Furthermore, Platt and Lu⁴ reported finding definite increases of bisulfite-binding substances, consisting chiefly of pyruvic acid, in the body fluids of persons with beriberi. From these and numerous other observations it is at once clear that the accumulation of pyruvic acid in the tissues and body fluids may be regarded as a specific "biochemical lesion" produced by a lack of vitamin B₁, and Cowgill⁵ suggests that quantitative examination of blood for pyruvate might be made the basis of a biochemical method for detection of a state of vitamin B₁ deficiency. The present series of experiments were undertaken to test the validity of this suggestion, and more specifically to find a simple and yet reliable method for the diagnosis of subclinical and clinical deficiency states in both man and animals.

EXPERIMENTAL PROCEDURES

For this experiment 12 normal rats were used, 6 males and 6 females, and each from a different litter. The males averaged 249 Gm. in weight, and the females 169 Gm. At the age of two months the animals were put on a synthetic vitamin B₁-free diet having the following composition:

Sugar	4,700 Gm.
Devitaminized casein	1,500 Gm.
Autoclaved liver	1,500 Gm.
Crisco	1,700 Gm.
Salt	250 Gm.
Cod-liver oil	300 c.c.

Each animal was kept in a separate wire cage with free access to a supply of distilled water contained in a clean inverted glass bottle. Daily inspection of general condition was supplemented by a semiweekly recording of body weight.

During the three days prior to the beginning of the devitaminized dietary regime, the blood pyruvate was determined in every animal, and the value so obtained was regarded as the normal basal level for that particular animal.

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During the entire period of experiment, extending over sixty days, the concentration of blood pyruvic acid was determined at weekly intervals. At the peak of avitaminosis, as evidenced by a pronounced increase in blood pyruvate and by the appearance of paralytic symptoms, a single dose (30 international units) of crystalline thiamin hydrochloride in aqueous solution was injected intraperitoneally. The changes in the level of blood pyruvic acid were then followed at intervals of two, four, six, eight, and twenty-four hours following the injection.

In order to ascertain the possible effect of inanition on the concentration of blood pyruvate, another group of 6 healthy rats, 3 males and 3 females, was put on an absolute starvation regime, allowing only an adequate supply of distilled water. The determinations of pyruvic acid in the blood of these animals were made at the end of twenty-four, forty-eight, and seventy-two hours. No further examinations were attempted inasmuch as after two days of starvation the values remained unaltered.

The average normal values of blood pyruvate in rats were obtained from 30 normal animals, equally distributed between the two sexes, and of different ages and litters.

All blood samples for chemical analysis were obtained from a cut in the tip of the tail made with a sharp razor blade. Since exercise, muscular effort, and emotional excitement are known to influence the pyruvate picture, the animal was first wrapped in a piece of soft cloth and then placed in a small wooden box. A small circular opening was provided at each end of the box, the one in the front for ventilation and the other in the back for permitting the tail to protrude. The animal so placed in the box was allowed to rest for at least one hour before the blood was withdrawn.

CHEMICAL METHODS

From the time of the discovery of pyruvic acid by Berzelius⁶ in 1835, no less than sixty different methods for its identification and estimation have appeared in the literature, according to the reviews published by Wendel⁷ and by Lu.⁸ However, only a few of these are applicable to clinical usage for blood analysis, and of these the following are noteworthy:

(1) The bisulfite-binding substance (B.B.S.) method of Clift and Cook⁹ is based on the property of the carbonyl compounds to react to bisulfite. This reaction, however, is not specific for pyruvic acid, since methylglyoxal, acetaldehyde, formaldehyde, and other ketonic acids also give the same positive reaction.

(2) The nitroprusside method of Simon and Piaux¹⁰ is generally believed to be specific for pyruvic acid, but the procedure is unsuitable for accurate quantitative analysis in the opinion of Platt.¹¹

(3) The 2,4-dinitrophenylhydrazine method of Case¹² is based on the characteristics of pyruvic acid to form hydrazone, and its solution in alcoholic potassium hydroxide is determined colorimetrically against a standard. The method, earlier used by Nenberg and Kobel,¹³ and later modified by Peters and Thompson,¹⁴ is specific for pyruvic acid; but it necessitates the use of a large amount of blood, and the length of time required to complete such an analysis makes its employment inadvisable for clinical purposes, especially if one is working with small subjects.

(4) The recently reported method of Lu,^s essentially a rapid and micro-modification of the Case-Nenberg technique, has by trial been found to be not only specific for pyruvic acid but admirably suited as well for use in clinical laboratories, especially in infants' and children's hospitals. These advantages commend themselves to experimental work in which small animals (rats, rabbits, and guinea pigs) are used. This method has been employed throughout the present investigation, the principal steps of which follow:

From a fresh cut on the tip of the rat's tail approximately 0.2 c.c. of blood is obtained and quickly dropped into a centrifuge tube containing exactly 1 c.c. of a 10 per cent solution of trichloroacetic acid which has been previously weighed and kept at a low temperature in a vessel packed with finely shaved ice. After mixing the clotted blood thoroughly with the acid by means of a fine glass rod, the actual amount of blood is calculated by reweighing the test tube and comparing its present weight with that prior to introduction of the blood.

Complete precipitation of blood proteins is allowed to take place at this low temperature for fifteen to twenty minutes, and then the tube is centrifuged for five minutes at high speed (1,500 to 2,000 r.p.m.). The clear supernatant fluid containing pyruvic acid is carefully transferred to a clean test tube (tube 1) by means of a long glass pipette. The precipitate is again treated with 1 c.c. of a 5 per cent solution of trichloroacetic acid and, after reagitation, is allowed to stand for five minutes at low temperature to remove the remaining portion of pyruvic acid. The supernatant fluid obtained after centrifugation is added to the contents of tube 1.

One cubic centimeter of a 0.1 per cent solution of 2, 4-dinitrophenylhydrazine solution in 2 N hydrochloric acid is next added to tube 1 and allowed to react at room temperature for at least ten minutes, for the complete formation of a hydrazone of pyruvic acid. The resulting hydrazone is then extracted with 2 c.c. of ethyl acetate, by first aspirating the latter up into the pipette and then expelling it into the layer of hydrazone at the bottom of the tube. By repeating the process several times a final homogeneous turbid mixture will be obtained. After standing a few minutes the bottom layer is then pipetted off into tube 2, the remaining acetate solution being kept in tube 1.

Another 2 c.c. portion of ethyl acetate is then added to tube 2 and extraction of hydrazone is repeated as before. The bottom layer, which becomes water-clear upon complete separation of the two layers, is discarded, and the remaining acetate solution is carefully transferred to tube 1.

The inner surface of tube 2 is rinsed with 2 c.c. of a 10 per cent solution of sodium carbonate, and the contents of the tube are then poured into tube 1. By means of the same pipette the top layer of the acetate is thoroughly mixed with the bottom layer of sodium carbonate. When the two layers are completely separated, the bottom layer is transferred back to tube 2. The same procedure for extracting pyruvic acid with sodium carbonate solution is repeated for the second and third times, the sodium carbonate layer being each time transferred to tube 2. The final acetate remaining in tube 1 is discarded, and the three extractions of pyruvic acid in sodium carbonate in tube 2 now total 6 c.c. in volume.

The final removal of a trace of hydrazone of ketonic acids other than pyruvic acid is made by the addition of 1 c.c. of ethyl acetate to tube 2. When the two layers are clearly separated, the yellow-colored bottom layer is carefully transferred to tube 3.

Four cubic centimeters of a normal sodium hydroxide solution are added to tube 3. The reddish color which then develops almost instantaneously will fluctuate somewhat if any impurities happen to be present in the mixture; and if so, color due to substances other than pyruvic acid will disappear within ten minutes. While allowing these ten minutes to elapse, the photoelectric cell colorimeter (Evelyn) is in the meantime being warmed to its optimal working condition. The unknown solution is then transferred to a colorimeter cell and, using filter 520, a galvanometer reading is recorded. The amount of pyruvic acid is calculated from a standard curve previously prepared by calibration of pure solutions of pyruvic acid (redistilled three times) in triple distilled water, in accordance with the directions given by Lu.⁸

RESULTS OF ANALYSES

The data obtained from blood analyses by the micromethod of Lu, as outlined and presented in Table I and Charts 1 and 2, comprise: (1) normal pyruvic acid values in the blood of rats, (2) pyruvate picture in various stages of vitamin B₁ deficiency, (3) blood pyruvic acid determinations in starvation, and (4) effect of thiamin on blood pyruvate in vitamin B₁-deficient rats.

As seen from Table I, the average normal value for blood pyruvic acid in 30 healthy rats, equally distributed between the two sexes, and ranging in age from two months to eight months, was 0.91 mg. per cent for the entire group. The maximum values were from 1.20 mg. per cent for females and 1.30 mg. per cent for males; the minimum figures varied from 0.50 mg. per cent for females to 0.60 mg. per cent for males. The standard deviations, as calculated from the equation, $D.S. = \sqrt{\frac{\sum m^2 - M^2}{N}}$, were ± 0.03 for females and ± 0.05 for males.

The data presented in Chart 1 depict the fluctuations in concentrations of blood pyruvate in 12 experimental animals at various stages of vitamin B₁ deficiency. The basal values in these animals were obtained within the three days prior to initiation of vitamin deprivation. That pyruvic acid progressively accumulates in the blood in direct proportion to the increasing severity of the deficiency state is evident. So much so, in fact, that by the end of the eighth week of observation, and coincident with the development of neurologic signs, the increase amounted to almost 500 per cent of the basal value.

One complicating factor affecting the pyruvic acid level of the blood in avitaminous rats is the influence of inanition, since in the majority of experimental animals on vitamin B₁-deficient diet anorexia was usually pronounced. In order to ascertain the effect of starvation, 6 healthy rats (3 males and 3 females) were deprived of all food and given a generous supply of distilled water. Determinations of blood pyruvate were made at the end of twenty-four, forty-eight, and seventy-two hours following the institution of starvation regime. From the data so obtained, and presented in Table I, it is clear that

inanimation produces a moderate increase in blood pyruvic acid values. These findings confirm the observations recorded by Lipschitz, Potter, and Elvehjem,¹⁵ who found the liver's ability to remove added pyruvate to be seriously impaired in fasting.

TABLE I
CONCENTRATION OF BLOOD PYRUVATE IN STARVATION
(3 MALES AND 3 FEMALES)

HOURS OF STARVATION	BLOOD PYRUVATE (MG. %)
24	2.43
48	3.15
72	2.70

The remarkably rapid and decisive influence of a single intraperitoneal injection of thiamin hydrochloride, in the calculated minimal dose (30 international units) for producing a state of saturation as recommended by Leong,¹⁶ is brought out in the data summarized in Chart 2. As revealed by these data, the action of thiamin in these animals is reflected within two hours after the injections, the blood pyruvate values dropping promptly to the extent of about 50 per cent of the preinjection level. At the end of four hours following injections, general condition of the animals was markedly improved, yet in each animal at this stage the blood pyruvate values were definitely elevated. This phenomenon may be explained on the ground that thiamin produces greatly accelerated activity of the animal. Increased activity, in turn, requires a more adequate liberation of phosphorylated thiamin, or cocarboxylase, by the cells in order to cope with the increased metabolism of the ketonic acid. By the eighth hour following injections the blood pyruvate usually had dropped nearly 65 per cent. One animal of this group, which failed to exhibit this typical response to thiamin at this stage, died about ten hours later. Platt¹¹ reported a similar observation in some cases of human beriberi. At twenty-four hours after injections the pyruvic acid of the blood remained at practically the same low level as in the previous examination. The clinical improvement was also remarkable, the only residual signs of deficiency being emaciation and spasticity of limbs.

DISCUSSION

According to Barron,¹⁷ pyruvic acid is an extremely reactive substance, having possibilities of undergoing at least fifteen different biochemical reactions, either with or without the catalytic action of thiamin and its pyrophosphoric ester. Thus the reactions of this ketonic acid, as characterized by reduction, couple oxidation-reduction, amination and transamination, proceed without intervention of vitamin B₁, whereas reactions such as oxidation, dismutation, and decarboxylation require its presence. From the work of Barron and Lyman¹⁸ the following statements may be quoted: "Under optimal conditions for oxidation, the pyruvic acid is directly oxidized to acetic acid and CO₂; under optimal conditions for reduction it may be reduced to lactic acid or may be split by dismutation into acetic acid and formic acid. . . . The direction and the extent of these reactions will be determined by the value of the constants of the equilibrium reactions taking place under the conditions of the experi-

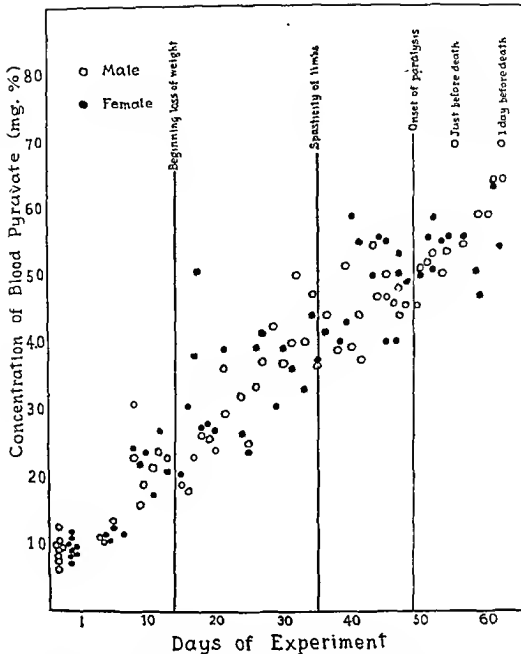


Chart 1.—Scattergram showing increasing values of blood pyruvate in direct proportion to progress of deficiency in rats.

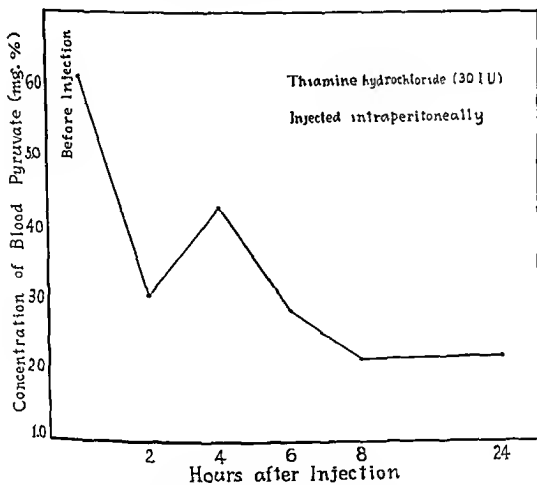


Chart 2.—Effect of intraperitoneal injection of thiamine hydrochloride on blood pyruvate level in vitamin B₁-deficient rats.

ment." These significant statements reveal the fundamental principle by which vitamin B₁ therapy should be applied in clinical practice.

While as yet no single system of glycolytic cycle has been satisfactorily established by either in vivo or in vitro experiments, pyruvic acid is now generally looked upon as a normal intermediary product of carbohydrate metabolism. It is formed by the splitting of the glucose molecule with the mediation of diphosphopyridine nucleotide during the process of glycolysis as well as in alcoholic fermentation. The degradation of pyruvic acid so formed proceeds by means of the catalytic action of thiamin in its phosphorylated form, known as cocarboxylase or diphosphothiamin. From recent investigations of Goodhart and Sinclair¹⁹ it appears clear that vitamin B₁ circulates in plasma in the form of a free unphosphorylated thiamin which diffuses readily into the cells and passes out into the tissue fluid. Phosphorylation of the vitamin with the formation of cocarboxylase occurs within the cells. In the blood there is but little free vitamin B₁ or cocarboxylase, most of it being combined probably with specific protein, according to the work of Sinclair.²⁰ Normal human blood contains an average of 7.0 micrograms per cent of carboxylase, mostly in combined form.²¹ In the absence or in the definite diminution of this vitamin, therefore, the removal of pyruvic acid is greatly retarded. This retardation in the rate of pyruvate disposal is responsible for its accumulation within the tissues and body fluids; the resulting "pyruvic acid acidosis," which is a specific biochemical lesion of avitaminosis B₁, is a measure of this deficiency.

Thus for diagnosing a possible vitamin B₁ deficiency state a direct estimation of either thiamin or cocarboxylase is logically the accurate measure. However, owing to the fact that the actual amount of thiamin or of diphosphothiamin present in the blood is small, and especially so in avitaminosis, the direct measurement is more difficult to perform than is the indirect estimation made by determining the concentration of blood pyruvic acid. In fact, the data obtained from animal experimentation here presented conclusively demonstrate pyruvic acid determination to be a simple and yet reliable measure of vitamin B₁ deficiency. By this means not only the accurate diagnosis of the clinical type of deficiency is possible, but also the identification of the subclinical type is easily demonstrable. The clinical application of this procedure for the diagnosis of vitamin B₁ deficiency in human beings will be reported in a subsequent communication.

SUMMARY AND CONCLUSIONS

The accumulation of the bisulfite-binding substances, particularly pyruvic acid, in the body fluids of avitaminous animals appears to play a significant role in the pathogenesis of symptom complex associated with vitamin B₁ deficiency. The present investigation was undertaken to ascertain whether or not estimation of pyruvic acid in the blood, rather than direct determination of thiamin or its cocarboxylase, could be used as a suitable method for the diagnosis of subclinical as well as clinical vitamin B₁ deficiency.

The results of chemical analyses for pyruvic acid in the blood of experimental animals (rats) by the use of the specific micromethod (2,4-dinitrophenylhydrazine) as reported by Lu⁸ indicate that by the end of the first

month of thiamin depletion the amount of blood pyruvate increased more than 260 per cent (3.49 mg. per cent) over the normal basal level (0.96 mg. per cent). During the second month the increase was almost 500 per cent (5.62 mg. per cent). Shortly before death there was usually a remarkable accumulation of pyruvic acid in the blood of thiamin-deficient animals. The magnitude of blood pyruvate appears to correspond roughly with the severity of clinical manifestations, since during the first month the only noticeable signs of avitaminosis were loss in weight, weakness, and anorexia; but during the second month definite neurologic symptoms began to develop. Upon injections of thiamin hydrochloride in these polynuritic animals a prompt fall in blood pyruvate occurred. If the injection of thiamin is not followed by this characteristic drop in blood pyruvate, death within twenty-four hours may be expected.

REFERENCES

1. Thompson, R. H. S., and Johnson, R. E.: Blood Pyruvate in Vitamin B₁ Deficiency, *Biochem. J.* 29: 694, 1935.
2. Johnson, R. E., Meiklejohn, A. P., Passmore, R., and Thompson, R. H. S.: A Note on the Level of Carbonyl Compounds in Human Blood, *Biochem. J.* 29: 2506, 1935.
3. Peters, R. A.: The Biochemical Lesion in Vitamin B₁ Deficiency, *Lancet* 1: 1161, 1936.
4. Platt, B. S., and Lu, G. D.: Intermediate Carbohydrate Metabolism in Vitamin B₁ Deficiency in Man, *Proc. Physiol. Soc.*, 3rd General Congress, Chinese Med. Ass., 1935; Chemical and Clinical Findings in Beriberi With Special Reference to Vitamin B₁ Deficiency, *Quart. J. Med.* 29: 355, 1936.
5. Cowgill, G. R.: The Physiology of Vitamin B₁, *J. A. M. A.* 110: 805, 1938.
6. Berzelius, J. J.: Ueber die Destillationsprodukte der Traubensäure, *Ann. Physik u. Chem.* 36: 1, 1835.
7. Wendel, W. B.: The Determination of Pyruvic Acid and the Preparation of Lithium Pyruvate, *J. Biol. Chem.* 94: 714, 1932.
8. Lu, G. D.: Studies on the Metabolism of Pyruvic Acid in Normal and Vitamin B₁ Deficiency States. A Rapid, Specific and Sensitive Method for Estimation, *Biochem. J.* 33: 249, 1939.
9. Chitt, F. P., and Cook, R. P.: A Method of Determination of Some Biologically Important Aldehydes and Ketones, With Special Reference to Pyruvic Acid and Methylglyoxal, *Biochem. J.* 26: 1788, 1932.
10. Simon, L. J., and Piaux, L.: Sur la caractérisation et dosage de petites quantités d'acide pyruvique, *Ann. biol.* 6: 477, 1924.
11. Platt, B.: Especially of Pyruvic Acid, in Relation to Some Clinical Conditions, *Roy. Soc. Trop. Med. & Hyg.* 31: 493, 1938.
12. Case, E. M.: The Determination of Pyruvic Acid, *Biochem. J.* 26: 753, 1932.
13. Neuberg, C., and Kobel, M.: Die Bildung von Brenztraubensäure als Durchgangsglied bei der alkoholischen Zuckerspaltung. Ihre Isolierung als Hauptprodukt der Gärung, *Biochem. Ztschr.* 216: 493, 1929.
14. Peters, R. A., and Thompson, R. H. S.: Pyruvic Acid as an Intermediary Metabolite in the Brain Tissue of Avitaminous and Normal Pigeons, *Biochem. J.* 28: 916, 1934.
15. Lipschitz, M. A., Potter, V. R., and Elvehjem, C. A.: The Metabolism of Pyruvic Acid in Vitamin B₁ Deficiency and in Inanition, *J. Biol. Chem.* 123: 267, 1939.
16. Leong, P. C.: Vitamin B₁ in the Animal Organism; the Maximum Storage of Vitamin B₁ in Various Species, *Biochem. J.* 33: 1394, 1939.
17. Barron, E. S. G.: Thiamine and Sulfhydryl Reactions, Symposium on Tissue Respiration, Ann. Meet., Fed. Am. Soc. Exper. Biol., New Orleans, May 15, 1940.
18. Barron, E. S. G., and Lyman, C. M.: The Metabolism of Pyruvic Acid by Animal Tissue and Bacteria, *J. Biol. Chem.* 127: 143, 1939.
19. Goodhart, R., and Sinclair, H. M.: Deficiency of Vitamin B₁ in Man as Determined by the Blood Cocarboxylase, *J. Biol. Chem.* 132: 11, 1940.
20. Sinclair, H. M.: The State of Vitamin B₁ in Blood, *J. Soc. Chem. Ind.* 57: 471, 1938.
21. Goodhart, R. S., and Sinclair, H. M.: Estimation of Cocarboxylase (Vitamin B₁, Diphosphate Ester) in Blood, *Biochem. J.* 33: 1099, 1939.

THE ABSORPTION OF NICOTINE FROM CIGARETTE SMOKE*

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ALTHOUGH many papers reporting work on the composition of cigarette smoke have been published, the question of the exact amount of nicotine absorbed in the smoking of a cigarette has, for the most part, escaped attention. Sollmann¹ states, "Between 15 and 90 per cent of the nicotine present in the tobacco are recovered from the smoke but a greater part of this is exhaled or expectorated." This statement apparently refers to pipe or cigar smoking, for in the same paragraph the author says, "In cigarette smoking, where inhalation is commonly practiced, the percentage absorbed would be relatively high." To quote from Asherson,² "the average cigarette contains 19 mg. of nicotine; in the portion actually smoked, about 14 mg., of this about 7 mg. enters the mouth." Lehmann³ found that about 5 mg. of nicotine was absorbed when the smoke from one cigarette was deeply inhaled.

Since nicotine is extremely soluble in water and almost nonvolatile at body temperature, it would seem remarkable if it would escape being retained in a very high degree when brought in contact, as it is in the inhalation of cigarette smoke, with the very large surface of the respiratory tract. The purpose of this study is to ascertain the amount of nicotine absorbed from cigarette smoke under conditions as nearly as possible like those present in ordinary smoking.

EXPERIMENTAL

Three brands of cigarettes were used: No. 1, a popular, medium-priced American cigarette, had a nicotine content of 2.27 per cent. No. 2, a so-called Egyptian cigarette, had a nicotine content of 1.16 per cent. No. 3, hand-rolled cigarettes made from a low-priced granulated tobacco, had a nicotine content of 2.63 per cent. All nicotine contents are based on dry weight of tobacco.

An absorption train was set up consisting of six ammonia-absorption tubes containing tenth-normal sulfuric acid. The cigarette was placed in a glass holder on the intake end and smoked by applying suction by mouth at the other end so as to draw the smoke into the train. Suction was applied at intervals, approximating as closely as possible the frequency, duration, and depth of inspiration employed in natural smoking. Ten aspirations were employed, since observation of smokers indicated that most of them draw about ten times while smoking a cigarette. That the aspirations were quite uniform was shown by the fact that the nicotine in the smoke from 5, 10, and 15 inspirations proved to be almost exactly in the ratio 1:2:3. The smoke thus inhaled is designated as the

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"main stream." In some experiments the smoke escaping from the burning tip was drawn off continuously through a second absorption train and is herein designated as the "side stream."

For determining the nicotine in the exhaled smoke, the following procedure was adopted: Air was drawn continuously through the train by a suction pump, and the smoke from the cigarette, after being deeply inhaled, was blown into the intake end. The exhaled smoke from ten deep inhalations was collected, the respiratory tract being ventilated after each exhalation by taking several deep breaths and exhaling into the train. In some experiments the smoke, instead of being deeply inhaled, was only drawn into the mouth, held for a few seconds and expelled. The nicotine absorbed is the difference between that in the main stream and that in the exhaled smoke.

Altogether, nicotine was determined in the cigarettes, the stubs, the main stream, the side stream, and the exhaled smoke. The cigarettes and stubs were extracted by the Kissling method, with final precipitation with silicotungstic acid. The smoke solutions were evaporated to a small volume, made alkaline, and the nicotine was distilled in a current of steam and precipitated with silicotungstic acid. Pyridine, in the amounts present in cigarette smoke, does not interfere.

DATA

There is some condensation of vaporized nicotine in the stub. The average nicotine content of the stubs of 50 cigarettes was 2.57 per cent, while that of the cigarettes was 2.27 per cent.

The distribution of nicotine between the stub, the main stream, the side stream, and that destroyed is shown in Table I.

TABLE I
AVERAGES FROM 20 CIGARETTES, BRAND NO. 1, 10 INHALATIONS
Nicotine in Milligrams

IN CIGARETTE	IN STUB	IN SMOKE		DESTROYED
		MAIN STREAM	SIDE STREAM	
27.43	11.91	3.88	4.31	3.83

The distribution is, of course, affected by tightness of rolling, moisture content, and the manner in which the tobacco is cut, as well as by the duration and force of aspiration.

The results of the experiments on absorption by deep inhalation are shown in Table II.

TABLE II
AVERAGES OF 50 CIGARETTES, 10 DEEP INHALATIONS

BRAND NO.	NICOTINE %	NICOTINE					
		IN CIGARETTE (MG.)	IN STUB (MG.)	IN INHALED SMOKE (MG.)	IN EXHALED SMOKE (MG.)	ABSORBED	
						MG.	IN % OF NICOTINE INHALED
1	2.27	23.2	12.27	3.74	0.17	3.56	95.0
2	1.16	11.65	6.16	2.76	0.15	2.61	91.6
3	2.63	25.1	14.75	2.60	0.32	2.28	87.8

When the smoke is not deeply inhaled, but only drawn into the mouth and expelled after two seconds, the absorption is distinctly less, as shown in Table III.

TABLE III

AVERAGES OF 50 CIGARETTES, BRAND NO. 1, SMOKE DRAWN INTO MOUTH ONLY AND EXPELLED AFTER 2 SECONDS. 10 INHALATIONS

NICOTINE			
IN INHALED SMOKE (MG.)	IN EXHALED SMOKE (MG.)	ABSORBED	
		IN MG.	IN % OF NICOTINE INHALED
3.74	0.85	2.89	77.3

The absorption of total solids was determined in the same manner as was that of nicotine, except that the smoke was absorbed in tenth-normal hydrochloric acid, the solutions evaporated to dryness, and the residues weighed. The results appear in Table IV.

TABLE IV

ABSORPTION OF TOTAL SOLIDS. AVERAGE OF 20 CIGARETTES, BRAND NO. 1, 10 DEEP INHALATIONS

IN INHALED SMOKE (MG.)	IN EXHALED SMOKE (MG.)	ABSORBED	
		IN MG.	IN %
26.4	8.4	18.0	68.2

DISCUSSION

These results show that of the nicotine present in the burned tobacco, about one-third is drawn into the mouth when a cigarette is smoked in the manner employed in these experiments. This figure probably represents the maximum amount, for the inspirations were as deep as possible to secure uniformity.

These experiments do not bear out the general impression that only a small fraction of the nicotine inhaled is absorbed. They show, on the contrary, that over 90 per cent is absorbed when the smoke is deeply inhaled and not much less when it is only drawn into the mouth.

The smoking of one package of 20 cigarettes of brand No. 1, under the conditions of these experiments, represents the absorption, with deep inhalation, of 71.4 mg. of nicotine, or without the inhaling of the smoke into the lungs, of 57.8 mg. If a smoker consuming one package of cigarettes a day can continue the absorption of this amount of nicotine over a long period of time without deleterious effects, it indicates either the establishment of a very high degree of tolerance to the effects of nicotine or that the toxicity of this alkaloid in the human being is very much lower than that indicated by minimum lethal doses as determined on animals.

The high degree of absorption of total solids is also of importance. These solids contain, besides nicotine, resinous and tarry substances, such as phenols, cresols, creosols, etc., ammonium salts, and other constituents in small amount. These undoubtedly contribute to the irritating effect of the smoke.

SUMMARY

When cigarette smoke is inhaled deeply, over 90 per cent of the nicotine in the smoke is retained in the respiratory tract.

If the smoke is not inhaled, but is drawn only into the mouth and expelled, about 77 per cent is retained.

The total solids of the smoke are retained to the extent of about 68 per cent.

REFERENCES

1. Sollmann, T.: *A Manual of Pharmacology*, ed. 5, Philadelphia, 1936, W. B. Saunders Co., Habitual Use of Tobacco, p. 394.
2. Asherson, N.: Nicotine and Tobacco Smoking, *Chem. News* 120: 150, 1920.
3. Lehmann, K. B.: Chemische und toxicologische Studien über Tabak, Tabakrauch und das Tabakrauchen, *Arch. f. Hyg.* 68: 321, 1909.

THE APPEARANCE OF BLOOD BROMIDE AFTER ORAL INGESTION*

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THE enactment of the Pure Food and Drug Law has brought many problems not only to the Government Bureau in charge of the enforcement of the act, but also to the manufacturers of self-medication products. Outstanding among these problems are those concerned with recommended doses and proper warnings as to possible dangers which may arise from incorrect use of many of the self-medication products on the market. Among the drugs which have received attention are the bromides, since they are contained in many preparations for the relief of minor headaches and pains.

Ten to fifteen grains of bromide several times a day has been considered a safe therapeutic dose when taken under the supervision of a physician. In epileptic cases as much as 10 to 15 Gm. have been given over a period of years without any untoward effects. These large doses are not given at the beginning of the treatment but are attained by gradually increasing the daily dose. It is generally recognized that a rash may appear from the use of bromides in allergic persons. This rash apparently has no relationship to the dosage taken and disappears rapidly when the medication ceases. However, the rash, together with persistent drowsiness, has been suggested as an early warning that the medication should be stopped.

It was to determine the effects of daily doses over a period of weeks that the present study was undertaken. It is stated in the literature that no harmful effects appear in bromide treatment until the blood bromides reach 200 mg. per hundred cubic centimeters of blood. Because of this theory, we used the blood bromide as an indication of the possible danger that might arise from taking the drug. In our study we used sodium bromide entirely, although both the ammonium and potassium salts are frequently employed. We are reporting on these in a later paper.

A group of 70 adults, ranging from ages 21 to 61, volunteered for our study. To assure that they would remain faithful to the study which lasted

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five months, they each received a nominal monthly fee and at the end of the study took an oath before a notary that they had carried out all of our instructions. No attempt was made to obtain only healthy persons in accepting the volunteers. The group contained several alcoholics. We felt that it was a representative cross section of the population. It consisted of college students, housewives, laboratory assistants, laboratory workers, clerks, artists, artist models, watchmen and janitors, and doctors.

Each individual received a careful physical examination at the beginning of the study, and at the end of the second and fourth months by a competent medical man who had no contact with them at any other time. Special attention was paid to the following factors: nervous reactions; reaction time to light signals; electrocardiogram; oxygen capacity; carbon dioxide capacity; hemoglobin; bromides in blood at the end of two, four, and five months, respectively; urine bromides; and changes in the white and red blood cell counts. The blood gases and hemoglobin were determined by the Van Slyke method.

For the determination of bromides we adapted the method of Katzenelbogen and Goldsmith,¹ based on the reaction of brown gold chloride with bromide, the quantity being estimated colorimetrically. Certain modifications were made in the quantities of reagents used. Four cubic centimeters of blood were added to 7 c.c. of 0.75 per cent sodium chloride solution and 3.4 c.c. of 20 per cent trichloroacetic acid and allowed to stand for one-half hour. The solution was then filtered. One cubic centimeter of a 0.5 per cent acid brown gold chloride solution was added to 5 c.c. of the filtrate and mixed well. The color was read in the Zeiss Pulfrich Photometer with a green Zeiss filter S53. The quantity of bromide present in 100 c.c. of blood was read from a curve derived from known blood bromide solutions. From time to time we checked the results by the Brodie and Friedman² method. The gold bromide method was purposely used because practically all the results reported in the medical literature are obtained by the Wuth method. It is conceded that this Wuth method is not a scientific method and does not give absolute results, but it is a good clinical method within certain ranges.

We divided the group into two sections: one consisting of 55 persons taking a total of 30 grains of bromides three times daily, the other consisting of 15 persons taking 15 grains three times daily. The bromides were taken each day, seven days a week for four months, and then stopped. The persons were examined at the end of the fifth month to see whether there was any difference in their physical condition and how much bromide still remained in the blood.

Since we could not detect any significant changes in the subjects, we are tabulating in Table I only our average findings.

A glance at Table I shows that there were no changes in the blood gases or blood cell counts. The same can be said for pulse rate and blood pressure. The electrocardiograms were negative throughout the study. The reaction time to light stimulation did not show any increase. In the case of the students we were able to obtain a record of their standing before the test began and at the end. These records showed that there was no lessening of their intellectual capacity; to the contrary there was a slight improvement. It is hard to make such a comparison, for the courses vary in different terms and one term's work may be easier than another.

TABLE I
AVERAGE FINDINGS

Group Taking 30 Grains of Sodium Bromide Daily								
	Pulse	Blood Pressure						
Beginning	76	132/81						
2nd month	72	131/77						
4th month	72	135/77						
Group Taking 45 Grains of Sodium Bromide Daily								
	Pulse	Blood Pressure						
Beginning	80	124/77						
2nd month	79	117/77						
4th month	85	119/77						
Group Taking 30 Grains of Sodium Bromide Daily								
	Bromides							
	O ₂ Cap.	CO ₂ Cap.	Van Slyke Hb.	W.B.C.	R.B.C	Urine Mg./ 100 c.c.	Blood Mg./ 100 c.c.	Chlo- rides
Beginning	19.33	62.7	14.49	7,892	4,810,000	Traces	Traces	-----
2nd month	19.25	63.0	14.44	7,355	4,960,000	29.11	22.44	446.67
4th month	19.29	63.0	14.48	7,831	4,973,000	13.67	16.55	443.51
5th month							Traces	442.21
Group Taking 45 Grains of Sodium Bromide Daily								
	Bromides							
	O ₂ Cap.	CO ₂ Cap.	Van Slyke Hb.	W.B.C.	R.B.C.	Urine Mg./ 100 c.c.	Blood Mg./ 100 c.c.	Chlo- rides
Beginning	20.0	63.0	14.9	8,437	4,743,000		Traces	467.21
2nd month	20.1	63.8	15.0	8,477	4,708,000	10.99	39.09	440.04
4th month	19.9	63.4	14.8	9,146	4,695,000	10.18	50.09	434.50
5th month							1.88	

Twenty-five of the group taking 30 grains daily over the four-month period were given a very careful examination by three well-known neurologists immediately after the medication had ceased. They pronounced the group to be normal in every way and expressed the opinion that they could serve as a control group in any study we would want to make.

No reports of drowsiness were received from the groups, and in only two persons was a rash observed. In one of these the rash was furunculosis, and in the other, a young lady, it was acne; it was rather hard to say whether this was due to the bromide or to the warm weather since the examination took place in August.

Our most interesting observation was the wide variation in the amount of bromides found to be present in the blood of various individuals. In some individuals this was approximately normal and in others it was much higher. The highest quantity found in our subjects was 89.6 mg. per 100 c.c. of blood at the end of four months. The average in the group taking 30 grains daily was 16.55 mg. In the group whose daily dosage was 45 grains, it was 50.09 mg. The same individual variations were noted in our animal experiments which are being reported in another paper. In the case of the smaller dose it would appear that the organism soon learns to handle the bromide and the blood level settles down to a more or less constant quantity. The individual differences may be accounted for by the variations in the amounts of sodium chloride taken by the individuals. It has been estimated in heat studies that the daily ingestion of salt varies from

TABLE II
GROUP TAKING 30 GRAINS OF SODIUM BROMIDE DAILY

NUMBER OF SUBJECT	TIME MEDICATION TAKEN	SAMPLE TAKEN NEXT MORNING BETWEEN 9:00 A.M. AND NOON	
		BLOOD BROMIDES (MG./100 C.C.)	URINE BROMIDES (MG./100 C.C.)
11	9:15 A.M.	34.6	46.0
7	7:30 A.M.	57.1	62.0
17	7:00 A.M.	Traces	3.5
23	8:15 A.M.	56.0	60.0
29	8:00 A.M.	44.2	58.0
35	9:00 P.M.	Traces	9.5
41	8:00 A.M.	21.0	43.9
47	9:00 A.M.	19.6	53.8
53	8:30 A.M.	15.9	31.4
59	8:15 A.M.	28.0	53.5
65	8:30 A.M.	6.7	19.0
67	10:30 P.M.	49.8	60.0
71	8:45 A.M.	22.4	48.9
77	8:30 A.M.	3.92	47.1
87	8:15 A.M.	31.9	35.1
3	8:00 P.M.	47.4	65.5

TABLE III

SUBJECT	SAMPLE OF BLOOD TAKEN	BROMIDES MG./100 C.C.	SAMPLES OF URINE TAKEN	BROMIDES MG./100 C.C.
<i>3 Grams of Sodium Bromide Taken at 10:20 A.M.</i>				
R. L.	10:37 A.M.	Traces	9:10 A.M.	Traces
	11:37 A.M.	0.14	11:30 A.M.	0.1
	12:03 P.M.	0.19	12:05 P.M.	8.3
	12:32 P.M.	0.84	12:35 P.M.	10.0
	1:00 P.M.	1.60	1:05 P.M.	6.3
	1:37 P.M.	1.60	1:40 P.M.	6.0
	2:50 P.M.	1.96	2:55 P.M.	0.8
	3:30 P.M.	0.19	3:33 P.M.	Traces
	4:37 P.M.	Traces	4:40 P.M.	Traces
			Next morning	Traces
<i>3 Grams of Sodium Bromide Taken at 10:15 A.M.</i>				
R. F.	10:30 A.M.	Traces	9:05 A.M.	Traces
	11:30 A.M.	Traces	11:35 A.M.	Traces
	12:00 P.M.	Traces	12:05 P.M.	8.7
	12:30 P.M.	Traces	12:35 P.M.	7.9
	1:00 P.M.	Traces	1:04 P.M.	4.6
	1:30 P.M.	0.28	1:35 P.M.	Traces
	2:40 P.M.	0.30	2:45 P.M.	Traces
	3:35 P.M.	Traces	3:30 P.M.	Traces
	4:28 P.M.	Traces	4:30 P.M.	Traces
			Next morning	Traces

TABLE IV

Cat 1 1 hour	Original solution contained	310.50 mg. bromide
	Recovered at end of 1 hour	215.46 mg. bromide
	Bromide absorbed	95.04 mg. bromide, or 30.6 per cent
Cat 2 2 hours	Original solution contained	313.20 mg. bromide
	Recovered at end of 2 hours	153.80 mg. bromide
	Bromide absorbed	159.30 mg. bromide, or 50.9 per cent
Cat 3 1 hour	Original solution contained	315.00 mg. bromide
	Recovered at end of 1 hour	230.85 mg. bromide
	Bromide absorbed	84.15 mg. bromide, or 26.7 per cent

10 to 20 Gm. There may be some explanation for the variation by rate of absorption from the gastrointestinal tract. In order to determine whether the time at which bromide was taken previous to the drawing of the blood was a factor, careful notation was made when the last dose was taken before the blood was drawn. The blood was taken, as a rule, between 9:30 A.M. and noon. Table II gives the results of this observation.

To determine how soon bromide could be detected in the blood after it was ingested, two of our technicians each took 3 Gm. in one dose. Samples of blood were drawn at frequent intervals throughout the day and samples of urine were collected at the same time. The results, shown in Table III, were somewhat of a surprise to us; they also showed the individual variations.

Three cats were anesthetized with nembutal. The abdomen of each was opened and a segment of the intestine was tied off. A cannula was inserted in either end of the segment, and the segment was washed out with a 0.6 per cent sodium chloride solution. The saline solution was then removed. The lower end of the segment was clamped off and a measured quantity of 0.90 per cent sodium bromide solution was permitted to flow into its upper end. The upper end was then clamped off, and the intestine was placed back in the abdominal cavity. It was allowed to remain there for one hour in two cats, and for two hours in the third cat. The segments were then washed out and analyzed for bromide. The results are indicated in Table IV.

In discussing our study with physicians, we were told that they have noted the same type of variations among their patients. Our observations indicate that after the medication has continued for a few weeks, the blood bromide tends to drop to a lower level and remain there. It is necessary to increase the quantity of sodium bromide ingested to get a higher blood bromide. This has been definitely indicated in our animal work and will be reported soon.

SUMMARY

1. The ingestion of 30 to 45 grains of sodium bromide daily over a period of four months does not result in a high blood bromide.
2. The blood bromide drops rapidly after the medication stops.
3. The ingestion of 30 to 45 grains of sodium bromide daily over four months does not affect the blood picture.
4. The ingestion of 30 to 45 grains of sodium bromide daily over a four-month period does not affect the nervous reactions.

REFERENCES

1. Katzenelbogen, S., and Goldsmith, H.: The Value of the Bromide Test in Mental Illness. *Psychiatry*, 10: 1045, 1931.
2. Brodie, B. B., and Friedman, N. M.: The Biological Fluids, *J. Biol. Chem.* 124: 511, 1938.

HIPPURIC ACID LIVER FUNCTION TEST: INTRAVENOUS METHOD*

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OUR experience with the oral hippuric acid test for liver function in determining the status of the liver in hyperthyroidism has been reported.¹ The test revealed reduction in the liver function in a high proportion of cases. Of the 148 patients studied, only 18 gave a normal response. The degree of change in the liver function was in fair proportion to the severity of the hyperthyroidism as determined by the basal metabolic rate and the clinical opinion as to the necessity for one or two operations. The value of the hippuric acid test as a satisfactory method of determining the function of the liver has been accepted by many investigators.²⁻⁷

About a year ago, at the suggestion of Dr. Quick, we began using the intravenous modification of the hippuric acid test. The test consists of the intravenous injection of 1.77 Gm. of sodium benzoate which has been dissolved in 20 c.c. of water. The urine is collected at the end of one hour and the hippuric acid content is determined. The normal excretion has been determined to be 1.77 Gm. or 0.7 Gm. of hippuric acid equivalent of benzoic acid.

A recent report by Quick⁸ gives in detail the procedure as employed in his laboratory. The test has much in its favor because of its simplicity. The fact that only one hour is required is an improvement, since the oral method requires four hours. We have now used the test over 300 times with the patient suffering only the slightest reaction or discomfort. At times there has been a slight flushing of the face and occasionally a sense of burning in the arm, but no other ill effects have been noted. Many difficulties encountered in the oral test, such as nausea and vomiting, and possible lack of absorption of the sodium benzoate from the stomach are overcome in utilizing the intravenous route. Patients have hesitated to cooperate in running serial tests by the mouth method because of the prevalence of nausea. The intravenous test has been carried out daily on the same patient without complaints.

The results of the intravenous tests in 58 patients with hyperthyroidism are given in Table I. Taking above 0.7 Gm. of benzoic acid as normal, we find only 7 patients (12 per cent) having satisfactory liver function. The remaining patients had hippuric acid excretions distributed throughout a wide range, to as low as 0.1 Gm., which is one-seventh the normal, and indicative of serious liver impairment. No correlation was found to exist between the level of the basal metabolic rate and the degree of disturbance of the liver function, as was found in our previous study utilizing the oral test. The duration of the hyperthyroidism and the weight loss were not related to the hippuric acid excretion.

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Most significant in judging the clinical opinion of the toxicity of the patient with hyperthyroidism is the final conclusion of the medical and surgical staff as to the type of operation the patient will most likely tolerate. This opinion is reached by a correlation of observations on the patient which cannot be appraised statistically. It can be seen from the chart that as the liver function decreased the number of operations apparently increased. This observation held true in our previous study with surprising regularity. Therefore, in spite of the failure of the test to show a close relationship to the basal metabolism, duration of disease, and average weight loss, it seems to be grossly indicative of even a more important conclusion, and that is the number of operations to which the patient should be submitted. In this way the liver function in hyperthyroidism plays an informatory role in the general medical survey. As has been shown in our previous studies, the level of the liver function was in no way related to the postoperative reaction. The basis of the postoperative thyroid storm still remains unexplained. It probably is not the result of alteration in the liver function as has been suggested by some authors who call thyroid crisis a liver reaction (liver death).

TABLE I

LIVER FUNCTION IN HYPERTHYROIDISM: INTRAVENOUS HIPPURIC ACID TEST

RANGE OF EXCRETION (GM.)	NUMBER OF CASES	AVERAGE B.M.R.	AVERAGE DURATION OF DISEASE (MONTHS)	AVERAGE WEIGHT LOSS (POUNDS)	NUMBER OF PATIENTS AND OPERATIONS		
					ONE	TWO	THREE
Over 0.70	7	+12	23	17	4	3	
0.66 to 0.70	7	+14	23	28	4	3	
0.61 to 0.65	6	+18	7	30	2	4	
0.51 to 0.60	9	+11	8	21	4	5	
0.41 to 0.50	8	+17	20	14	2	6	
0.31 to 0.40	10	+52	8	21	2	8	
0.21 to 0.30	4	+48	7	16	2	1	1
0.11 to 0.20	7	+39	12	20	3	4	
Total	58						

Liver Function in Other Thyroid Conditions.—Patients with adenomatous goiter without hyperthyroidism showed normal hippuric acid excretions. In one patient with subacute thyroiditis with slight fever there was a slight reduction to 0.65 Gm. One patient with chronic myxedema had still greater reduction with an excretion of 0.42 Gm. One patient with therapeutic hyperthyroidism, who took 6 grains of desiccated thyroid a day in an effort to reduce weight, had an excretion which was a little more than one-third normal, 0.27 Gm. This patient shows evidences of the depleting effect of hyperthyroidism on the liver and also seems to indicate the fact that the test determines functional impairment since it is highly probable or almost certain that with the discontinuance of the desiccated thyroid the liver function in this patient will return to normal. This we determined to be true in clinical hyperthyroidism.

Primary Liver Disease.—In Table II the sample results of the test in diseases primarily affecting the liver are given. In all cases of cirrhosis of the liver a reduced function was found, in most instances to a marked degree. The same was true in the other cases of liver disease, which were the result of either in-

fection, chemical toxicity, or neoplastic infiltration. In catarrhal jaundice alone, which we do not recognize as producing intrinsic liver disease, the liver function was practically normal. Strangely enough, in three of four cases of chronic alcoholism, not recorded in Table II, in which no apparent physical or laboratory defects could be ascertained, the liver function (0.65, 0.64, 0.74, 0.67 Gm.) was only slightly altered in three instances.

TABLE II
LIVER FUNCTION BY INTRAVENOUS HIPPURIC ACID TEST
DISEASES OF THE LIVER

A. Cirrhosis	
Number of cases studied, 11	
Range of hippuric excretion	
0 to 0.40, 8 cases	
0.40 to 0.5, 2 cases	
	Hippuric acid excretion, Gm.
B. Cholangitis, 1 case	0.29
C. Primary carcinoma of liver, 1 case	0.16
D. Amoebic abscess of liver, 1 case	0.39
E. Arsphenamine hepatitis, 1 case	0.11
F. Catarrhal jaundice, 1 case	0.68
G. Metastatic infiltration of liver, 2 cases	0.40 to 0.12

Miscellaneous Diseases.—In a group of 5 patients with idiopathic splenomegaly, the test gave normal results varying from 0.68 to 0.88 Gm. In exogenous obesity equally normal results were obtained, irrespective of the degree of overweight. In chronic infectious arthritis there was a uniform reduction in the hippuric acid output to about 25 per cent. This reduction is apparently the result of the effect of the strain of detoxification on the liver.

One person with severe muscular dystrophy was tested, with a normal result, the excretion being 0.8 Gm. This result is significant as one criticism of the hippuric acid test has been the possible assumption that it may be the summation of the conjugation of sodium benzoate with glycine in both the liver and muscle. If this were true, a reduction in function should have resulted in this case.

The depleting effect of anesthesia on the liver is now under investigation. A number of investigators have reported the deleterious effect of various anesthetics on the liver. In the cases studied so far the hippuric acid excretion dropped 23 per cent on the day of the operation, with a gradual return toward normal on the third postoperative day.

CONCLUSIONS

1. The intravenous hippuric acid test of liver function has been used in a large group of patients without subjective or objective reactions. The simplicity of the test is noted. The results in a group of patients with hyperthyroidism compare favorably with the results previously reported in this condition in which the sodium benzoate was given by mouth.

2. In intrinsic liver disease the degree of reduction in the hippuric acid excretion is in direct accord with the clinical and autopsy findings as to the liver damage. This finding speaks favorably for the test.

3. The fact that repeated tests can be carried out without ill effects increases the value of the test.

REFERENCES

1. Bartels, E. C.: Liver Function in Hyperthyroidism as Determined by the Hippuric Acid Test, *Ann. Int. Med.* 12: 652, 1938.
2. Snell, A. M., and Plunkett, J. E.: Hippuric Acid Test for Hepatic Function; Its Relation to Other Tests in General Use, *Am. J. Digest. Dis.* 2: 716, 1936.
3. Yardumian, K., and Rosenthal, P. J.: Hippuric Acid Elimination as a Test for Liver Function, *J. LAB. & CLIN. MED.* 22: 1046, 1937.
4. Kohlstaedt, K. G., and Helmer, O. M.: Study of Hippuric Acid Excretion as Test of Hepatic Function, *Am. J. Digest. Dis.* 3: 459, 1936.
5. Vaccaro, P. F.: Synthesis of Hippuric Acid; Its Value in Detecting Hepatic Damage Secondary to Diseases of Extrahepatic Biliary System, *Surg., Gynec. & Obst.* 61: 36, 1935.
6. Boyce, F. F.: Hepatic and Biliary Tract Disease; Review of Recent Significant Advances, *Ann. Surg.* 109: 351, 1939.
7. Haines, S. F., Magath, T. B., and Power, M. H.: Hippuric Acid Test in Hyperthyroidism, *Proc. Staff Meet., Mayo Clin.* 14: 495, 1939.
8. Quick, A. J.: Intravenous Modification of the Hippuric Acid Test for Liver Function, *Am. J. Digest. Dis.* 6: 716, 1939.

THE HYPERPHOSPHATASEMIA OF PAGET'S DISEASE*

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THE serum phosphatase activity is characteristically increased in persons with Paget's disease of bone. Different opinions have been expressed with regard to the nature of this hyperphosphatasemia. For example, Kay¹ relates the high values to an excess of phosphatase in the blood due to the diffusion of the enzyme from the sites of formation in the bones at rates above the normal. On the other hand, Thannhauser and co-workers,^{2, 3} on the basis of their experiments, and the observations of Freeman and Chen,⁴ conclude that the increased serum phosphatase activity found in pathologic conditions, including Paget's disease, is due to the presence of an activator of the enzyme in the blood rather than to an actual increase of the amount of the phosphatase.

An exact study of the question was made possible by the work of O. Bodansky^{5, 6} on the relation of amino acids to phosphatase activity. He demonstrated that under optimal conditions of pH, amino acid, and magnesium ion concentrations, a measure of the reaction velocity of the hydrolysis of the substrate (sodium β -glycerophosphate) by bone phosphatase is proportional to the concentration of the enzyme present. It may be assumed, therefore, that the measure of the reaction velocity ($Q_{0.05}$) can be used to indicate the relative concentration of the phosphatase in the sera of persons with bone disease, especially since O. Bodansky⁷ showed also that the phosphatases of the bones and the blood probably are identical.

On the basis of this hypothesis, the experiments described herein were conducted on the blood sera of several patients with Paget's disease in an attempt to determine the relationship between the phosphatase activity and the quantity of the enzyme.

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PROCEDURES

The methods described by A. Bodansky⁸ for the estimation of the inorganic phosphorus and phosphatase activity were used with the application of all the suggested correction factors for the deviation from Beer's law, the presence of trichloroacetic acid and glycerophosphoric acid, retardation of hydrolysis, and conversion to a standard time of one hour.

The extraction of the phosphatase was accomplished by treating the sera in the following four ways:

1. The serum was diluted and dialyzed in a collodion membrane for eighteen hours against distilled water. The dialysate, usually clouded with precipitated protein which dissolved on the addition of a few drops of dilute alkali, was diluted to a definite volume, and aliquot portions were used to estimate the reaction velocity.

2. The phosphatase and much of the protein were precipitated by the ether-alcohol method of Martland and Robison.⁹ The precipitate was filtered off, washed with an ether-alcohol-water (3:2:1) mixture, and dispersed in a definite volume of water containing a few drops of a dilute alkali solution. Aliquots were used as above for the reaction velocity.

While both the foregoing procedures removed inorganic matter, such as magnesium ions, phosphate ions, amino acids, and hydrolyzable organic phosphorus compounds, a large proportion of the serum proteins remained.

3. Considering the serum as a solution of phosphatase, the latter was fractionally precipitated by 95 per cent ethyl alcohol, according to the method of Albers and Albers.¹⁰

4. The first precipitation was carried out as in procedure No. 3, but the 50 per cent alcohol filtrate and washings were treated with either a triple volume of 2:1 ether-alcohol mixture or a double volume of 3:1 ether-alcohol mixture, and the precipitate was filtered off. Thus better yields of phosphatase with a degree of purity equal to that of the other methods were obtained with the added advantage that the second filtration was more rapid. This method proved to be the most satisfactory.

Aliquots of the solutions of phosphatase obtained by the procedures outlined were added to hydrolysis mixtures each composed of substrate, buffer, magnesium chloride solution, glycine solution, and dilute sodium hydroxide solution.¹¹ The hydrolysis mixtures and the enzyme solutions were brought to 25° C. before mixing, and the hydrolysis was carried out at 25° C. in a constant temperature water bath thermostatically controlled.

Portions of the hydrolysis mixtures were removed at intervals after the beginning of the reaction. In experiments where the serum had been treated as in the first two methods, the 1 ml. aliquots were added to 7 ml. of 10 per cent trichloroacetic acid solution. The resulting precipitate was filtered off (Whatman No. 44), and the phosphorus in 6 ml. of the filtrate was estimated by the method of A. Bodansky.⁸ When the serum was treated as in the third and fourth procedures, no appreciable precipitate formed; hence the 1 ml. aliquots were added to 5 ml. of the trichloroacetic acid solution and the phosphorus was estimated without filtration.

The tables devised by A. Bodansky⁸ were converted for the purpose of the present calculations by means of the factor 0.006, so that his data thus modified gave at once the concentration of phosphorus per milliliter of the hydrolysis solution. The results were corrected also for loss on filtration when necessary. The final results were plotted against the time of the hydrolysis and the $Q_{0.05}$, as recommended by O. Bodansky,¹² was calculated. This value was then related to the amount of serum used originally.

If the phosphatase precipitated from 5 ml. of serum is dissolved in 25 ml. of water and 2 ml. of this solution is used in the hydrolysis mixture, then $Q_{0.05} \times \frac{25}{2 \times 5}$ gives what may be regarded as a measure of the reaction velocity per milliliter of serum. This numerical value is proportional to the concentration of the phosphatase in the serum.

RESULTS

The results are expressed as the serum phosphatase activity in Bodansky units and the reaction velocity per milliliter of serum as shown in Fig. 1. The first three methods of extraction gave similar values and are not differentiated (curve A). The results obtained by the fourth method are represented by curve B. From this chart it is evident that as the serum phosphatase activity in Bodansky units increases, the measure of the reaction velocity, and hence the concentration of the phosphatase per milliliter of serum, increases in direct proportion.

The high serum phosphatase values found in these patients with Paget's disease are associated, therefore, with an increased concentration of the enzyme. The lower results obtained for the reaction velocities in curve A are attributed to losses of phosphatase in the third method of extraction and to inhibition by the serum proteins in the first two methods.

Not only did the concentrations of the phosphatase in the sera vary directly as the activity in Bodansky units in general, but each individual result was well above the normal range for both the activity in Bodansky units and the reaction velocity per milliliter of serum. This is exemplified by comparing the results depicted in Fig. 1 with those of a group of normal sera shown in Table I.

TABLE I
NORMAL SERA

SERUM PHOSPHATASE ACTIVITY (BODANSKY UNITS)	RELATIVE CONCENTRATION OF PHOSPHATASE	SERUM PHOSPHATASE ACTIVITY (BODANSKY UNITS)	RELATIVE CONCENTRATION OF PHOSPHATASE
2.23	0.00083	3.36	0.00137
2.50	0.00105	3.43	0.00124
2.54	0.00103	3.61	0.00142
3.03	0.00134	3.67	0.00140
3.15	0.00138	4.02	0.00146

From these observations it may be concluded that the sera of normal adults contain less phosphatase than the sera of patients with Paget's disease and that the increased serum phosphatase activity of the latter is not due to the

removal of an inhibitor normally present in the blood or to the addition of an activator of the enzyme. The elevation of the serum phosphatase activity in cases of Paget's disease appears to be due rather to an actual excess of the enzyme in the blood stream.

The data in Table I seem to be sufficiently consistent to indicate that the variations in the phosphatase activity of normal sera as well as those of Paget's disease are due to variations in the concentration of the enzyme.

Preliminary observations concerning patients with other diseases, such as jaundice and arthritis, also suggest that in those cases in which the serum phosphatase activity is elevated, the elevation is related to an increase of the enzyme in the blood.

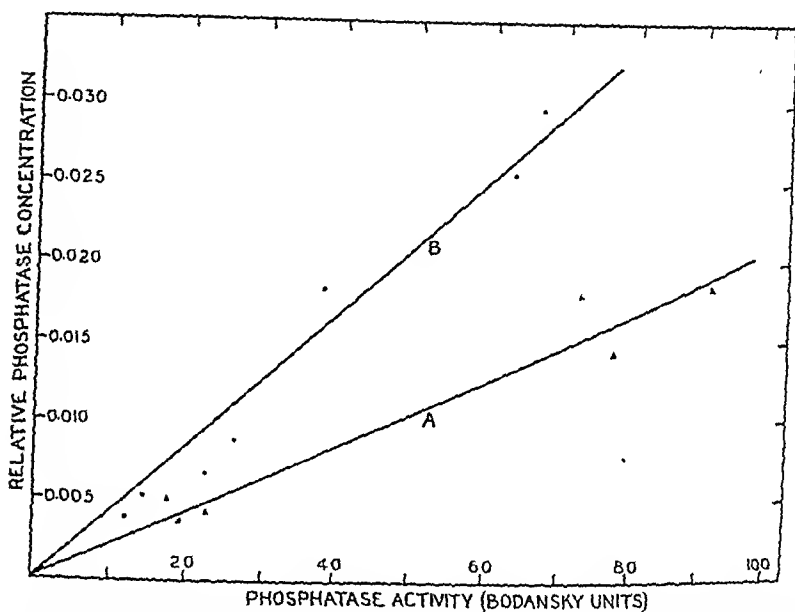


Fig. 1.—Chart showing the direct proportionality between the serum phosphatase activity in Bodansky units and the concentration of the enzyme per milliliter of serum.

COMMENT

The observations described in this report are at variance with those of Thannhauser and associates.^{2, 3} If the increase of serum phosphatase activity in Paget's disease is due to the presence of an activator of the enzyme in the serum as they suggest, the increased concentrations of phosphatase, as indicated by elevated reaction velocities, which we found, would not occur. In order that the increased activity be caused by an activator, the latter would have to be similar in chemical and physical properties to the enzyme molecule itself so that it would be inseparable from it by the methods used for the purification of the enzyme.

If the increase of serum phosphatase activity is due to the removal of an inhibitor of the phosphatase which is normally present in the blood, the concentrations of the enzyme in the sera of normal persons and those with Paget's disease, as indicated by the reaction velocities, would present similar values.

This is not the case, for not only are the values generally higher for both the serum phosphatase activity and the relative concentration of the phosphatase in the patients with Paget's disease, but each individual determination is greater than even the highest normal value.

SUMMARY

Methods are outlined whereby the concentration of the serum phosphatase is expressed in relation to a measure of the reaction velocity of the substrate under specific conditions.

According to the evidence presented, the hyperphosphatasemia of patients with Paget's disease of bone is due to an actual increase of the concentration of the phosphatase in the serum as opposed to the view that it is related to the presence of an activator of the enzyme in the blood.

REFERENCES

1. Kay, H. D.: Plasma Phosphatase. II. The Enzyme in Disease. Particularly in Bone Disease, *J. Biol. Chem.* 89: 249, 1930.
2. Thannhauser, S. J., Reichel, M., Grattan, J. F., and Maddock, S. J.: Studies on Serum Phosphatase Activity. VI. The Influence of Sera With High Phosphatase Activity on Normal Sera, *J. Biol. Chem.* 124: 631, 1938.
3. Maddock, S., Thannhauser, S. J., Reichel, M., and Grattan, J.: A New Conception of Serum Phosphatase, *New England J. Med.* 218: 166, 1938.
4. Freeman, S., and Chica, Y. P.: The Effect of Jaundiced Blood Upon Normal Dogs, With Special Reference to the Serum Phosphatase, *J. Biol. Chem.* 123: 239, 1938.
5. Bodansky, O.: The Effect of Alpha-Amino Acids and Magnesium on the Activity of Kidney and Intestinal Phosphatase, *J. Biol. Chem.* 115: 101, 1936.
6. Bodansky, O.: The Accelerant Effect of Alpha-Amino Acids on the Activity of Bone Phosphatase, *J. Biol. Chem.* 114: 273, 1936.
7. Bodansky, O.: Are the Phosphatases of Bone, Kidney, Intestine and Serum Identical? The Use of Bile Acids in Their Differentiation, *J. Biol. Chem.* 118: 341, 1937.
8. Bodansky, A.: Notes on the Determination of Serum Inorganic Phosphate and Serum Phosphatase Activity, *J. Biol. Chem.* 120: 167, 1937.
9. Martland, M., and Robison, R.: The Preparation and Use of the Bone Phosphatase, *Biochem. J.* 23: 237, 1929.
10. Albers, H., and Albers, E.: Über die Nierenphosphatase (1. Mitteilung zur Kenntnis der Phosphatasen), *Ztschr. f. physiol. Chem.* 232: 165, 1935.
11. Bodansky, O.: The Energy of Activation of the Hydrolysis of Sodium Beta-glycerophosphate by Bone Phosphatase at Optimum pH, *J. Biol. Chem.* 129: 197, 1939.
12. Bodansky, O.: The Use of Different Measures of Reaction Velocity in the Study of the Kinetics of Biochemical Reactions, *J. Biol. Chem.* 120: 555, 1937.

LABORATORY METHODS

GENERAL

THE PREPARATION OF ANTI-M, ANTI-N, ANTI-A, AND ANTI-B TESTING FLUIDS*

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TO PERFORM the simple blood-grouping test prior to transfusion, one must possess, as is well known, high titered anti-A and anti-B sera which are obtained ordinarily from particular group B and group A individuals, respectively. In a large hospital, where many blood-grouping tests are done daily, the supply of these two sera must always be adequate; or group A and group B individuals, whose sera are known to contain potent agglutinins, must always be at hand. However, group specific sera can also be prepared by immunizing rabbits with human blood and such immune anti-sera usually possess a high agglutinin titer, as shown by Hooker and Anderson,¹ Landsteiner² and von Dungern and Hirschfeld.³

Aside from the above blood group factors A and B, Landsteiner and Levine⁴⁻⁶ discovered other agglutinable properties in the human erythrocyte, which they called M, N, and P. (In this paper, factor P will not be discussed.) They found that every individual possessed either the factor M, N, or both M and N in addition to his blood group. To determine the presence of these agglutinogens M or N, immune rabbit serum was used since human agglutinins for M and N have not been found, aside from three rare exceptions.^{7a} An individual may be in the blood group O or A, or B or AB, and also belong to type M, N, or MN.

To produce these blood-group specific, immune anti-sera, the appropriate groups and types of human blood cells must be injected into rabbits. Various methods and techniques^{6, 7b-15} have been employed for the production of these immune anti-sera. Such sera have a wide practical application; namely, (1) checking doubtful reactions encountered in routine blood-grouping tests; (2) in medico-legal cases, the grouping of dried blood stains not too badly decomposed;^{16, 17} (3) exclusion of parentage;^{18, 19} (4) anthropologic studies;²⁰ (5) tracing the fate of the transfused red blood cell in patient's circulation.^{21, 22}

In this paper, I shall present my experiences with the preparation of anti-M, anti-N, anti-A, and anti-B immune rabbit sera, using Wiener's⁷ method of immunization. I do not claim that this procedure is superior to others.

PREPARATION OF ANTI-M AND ANTI-N TESTING FLUIDS

Anti-M.—In the preparation of anti-M serum, 8 rabbits were immunized with blood from an OM individual. OM cells were used to avoid the formation

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of anti-A or anti-B agglutinins. Sterile precautions were taken throughout the procedures so that the final testing fluids, after ampouling, would be free of any bacteria. A total of 25 c.c. of OM blood were withdrawn into 5 c.c. of sterile 3.8 per cent sodium citrate, and this was immediately divided into six equal parts, each representing the total daily dose for the 8 rabbits. Before injection the blood sample was washed free of all plasma with physiologic saline and then suspended in about 20 c.c. of saline. Each rabbit was given intravenously 2 c.c. of this red blood cell suspension. The sixth daily injection was followed by a rest period of seven to ten days; this may be considered the first course. The first injection of each subsequent course was given subcutaneously to avoid anaphylaxis, but all other injections were given intravenously. No tests were made until end of third course, since Wiener^{7b},* found that only rarely were good sera obtained in a shorter time.

TABLE I
PREPARATION OF ANTI-M SERA
PRELIMINARY TESTS

IMMUNE, INACTIVATED SERUM DILUTED 20 TIMES AND AFTER 2ND ABSORP- TION WITH POOLED N CELLS	KNOWN BLOOD CELL SUSPENSION			DILUTIONS OF TESTING FLUID FOR TITRATION AND TESTED AGAINST M CELLS						
	M	N	MN	1:1	1:5	1:10	1:20	1:40	1:80	TITER
Rabbit M1	+++	-	+++	+++	++±	++	±±	+	-	40
Rabbit M2	+++	-	+++	+++	++	±±	+	-	-	20
Rabbit M3	++	-	++	++	+	±	-	-	-	5
Rabbit M4	+++	-	+++	+++	++±	++±	++	±±	±	40
Rabbit M5	+++	-	+++	+++	++	±±	+	-	-	20
Rabbit M6	+++	-	+++	+++	++±	++	±±	+	-	40
Rabbit M7	+++	-	+++	+++	++±	++	±±	±	-	20

+++ complete agglutination.

- no agglutination.

Of the 8 rabbits injected with OM blood 7 remained alive, and from the marginal ear veins of these 7 rabbits about 5 c.c. of blood were withdrawn one week after the last injection of the third course. In addition to the anti-M agglutinins present, these 7 immune sera would also contain species agglutinins capable of agglutinating any human blood, and these so-called nonspecific antibodies must be removed by absorption with the proper human blood cells, in this case ON, AN, and BN bloods.* Accordingly, in the preliminary tests, each of the seven sera, after inactivation for one-half hour at 56° C., was diluted twenty times with saline, and to each now was added one-half volume of pooled, packed washed ON, AN, and BN blood cells, mixing thoroughly to insure as near a complete agglutination and absorption as possible. These tubes were left at room temperature for one-half hour, centrifuged, and the supernatant fluid tested against 2 per cent cell suspensions of OM, ON, and OMN blood. Since there was still a definite agglutination with ON cells, one-fourth volume of the pooled ON, AN, and BN cells was added to the supernatant fluid, and this second absorption removed completely the remainder of the species agglutinins. These seven fluids were then titrated against OM cells. Table I presents the reactions of the various sera in tests carried out in small test tubes at room temperature

*These known standard and controlled bloods are usually from doctors, nurses, or employees in the hospital and therefore are always available for absorption and testing purposes.

and read one hour after the set-up. The rabbits with a specific anti-M titer of 20 or more were then bled, their sera were inactivated at 56° C. for one-half hour, ampouled, and stored in an icebox. When anti-M testing fluid was needed, one of these ampoules was diluted twenty times with saline and absorbed with pooled N cells. This anti-M testing fluid was now ready for use.

Anti-N.—To prevent the formation of interfering agglutinins, anti-A and anti-B, ON blood was used for the injections, 8 rabbits being immunized by the technique already described. After the third course, 6 rabbits survived and their sera were tested, using pooled M cells for the absorptions. The same procedure was followed as outlined under "Anti-M," except that the sera were diluted fifteen times and the absorptions were carried out at 37° C. instead of room temperature. Three absorptions were necessary before all the species agglutinins were removed and the fluids were then titrated. In Table II it will be seen that the titers of the N agglutinins are lower than the titers of the M agglutinins (Table I), and only two of the anti-N sera were satisfactory for use.

TABLE II
PREPARATION OF ANTI-N SERA
PRELIMINARY TESTS

IMMUNE, INACTIVATED SERUM DILUTED 15 TIMES AND AFTER 3RD ABSORP- TION WITH POOLED M CELLS	KNOWN BLOOD CELL SUSPENSION			DILUTIONS OF TESTING FLUID FOR TITRATION AND TESTED AGAINST N CELLS						
	M	N	MN	1:1	1:2	1:4	1:8	1:16	1:32	TITER
Rabbit N1	-	+++	++±	+++	++±	++	++	+	-	16
Rabbit N2	-	++	++	++	++	+	±	-	-	4
Rabbit N3	-	++	++	++	±	+	±	-	-	4
Rabbit N4	-	+++	+++	+++	+++	++	±	+	-	16
Rabbit N5	-	++	++	++	++	+	±	-	-	4
Rabbit N6	-	++	++	++	+	-	-	-	-	2

PREPARATION OF ANTI-A AND ANTI-B TESTING FLUIDS

Anti-A.—In the preparation of anti-A serum, 10 rabbits were immunized with AN blood in a fashion similar to that described under "Anti-M." Four rabbits survived and from the marginal ear veins of these 4 rabbits about 5 c.c. of blood were withdrawn one week after the last injection of the third course. As AN blood had been injected, each rabbit could be expected theoretically to produce specific agglutinins for N as well as for A. It was thought that should the rabbits fail to manufacture a serum rich in anti-A agglutinins perhaps they would produce a usable anti-N serum, especially since our anti-N yield from the ON immunization was not large.

Accordingly, in the preliminary tests, each of the four sera, after inactivation, was diluted fifteen times with saline and divided into two portions: one portion to determine the content of anti-A agglutinins and the other portion the content of anti-N. To the first portion of each inactivated and diluted rabbit's serum was added one-half volume of pooled, packed, washed OM, BN, OMN, and BMN cells,* mixing thoroughly to insure as near as complete agglutination and absorption as possible. These tubes were left at room temperature for one-

See footnote () page 1339.

half hour and centrifuged, and the supernatant fluid was tested against 2 per cent cell suspensions of OMN, ON, AMN, and BMN bloods. These tests showed that the first absorption in most cases was not complete, so a second absorption was performed, this time with one-fourth volume of the packed, pooled, and washed cells.

TABLE III
PREPARATION OF ANTI-A SERA
PRELIMINARY TESTS

IMMUNE, INACTIVATED SERUM DILUTED 15 TIMES AND ABSORBED WITH OM, DN, OMN, AND DMN CELLS		KNOWN BLOOD CELL SUSPENSIONS				INTERPRE- TATION
		OMN	ON	AMN	DMN	
Rabbit A4	1st absorption $\frac{1}{2}$ volume	±	±	±±	+	Very weak anti-A fluid
	2nd absorption $\frac{1}{4}$ volume	-	-	+	-	
Rabbit A5	1st absorption $\frac{1}{2}$ volume	-	+	+++	-	Strong anti-A fluid
	2nd absorption $\frac{1}{4}$ volume	-	-	+++	-	
Rabbit A6	1st absorption $\frac{1}{2}$ volume	+	±±	+++	+	Strong anti-A fluid
	2nd absorption $\frac{1}{4}$ volume	-	-	+++	-	
Rabbit A7	1st absorption $\frac{1}{2}$ volume	-	+	±±	-	Weak anti-A fluid
	2nd absorption $\frac{1}{4}$ volume	-	-	±±	-	

As can be seen in Table III, rabbits 4 and 7 produced a very poor anti-A serum, but rabbits 5 and 6 formed a strong anti-A serum. To determine the potency of the anti-A agglutinins in the sera from rabbits 5 and 6, each diluted and absorbed serum was titrated against suspensions of AMN and BMN bloods, as outlined in Table IV.

TABLE IV
TITRATION OF ANTI-A TESTING FLUID

TESTING FLUID	TESTED AGAINST	DILUTIONS OF TESTING FLUID								TITER
		1:1	1:10	1:20	1:40	1:80	1:160	1:320	1:640	
Rabbit 5	AMN	+++	+++	+++	+++	++	±	-	-	80
	BMN	-	-	-	-	-	-	-	-	
Rabbit 6	AMN	+++	+++	+++	+++	±±	++	++	±	320
	BMN	-	-	-	-	-	-	-	-	

To determine the content of anti-N agglutinins in the sera of the 4 rabbits, the second portion of diluted inactivated serum was used. To each was added a one-half volume of pooled, packed, washed OM, AM, and BM red blood cells. After standing at 37° C. for one-half hour, the tubes were centrifuged, the supernatant fluids were drawn off and tested against AM, BM, ON, and OMN cell suspensions. It was noticed that the fluids from the sera of rabbits 4 and 7 gave strong agglutination reactions with ON and OMN cells, but only a slight reaction with AM and BM cells; while the fluids prepared from sera of rabbits 5 and 6 reacted poorly. A second absorption was carried out on the supernatant fluids, 4 and 7, this time using one-fourth volume of packed and pooled M cells.

After the second absorption, these two fluids were retested and found to be satisfactory for titration against N cells. The titer of the anti-N agglutinins was found to be approximately 16 (compare with Table II).

It will be seen that of the 4 rabbits immunized with AN blood, 2 produced potent A antibodies and very little N antibodies, while the other 2 produced satisfactory anti-N agglutinins and only weak A antibodies. These 4 animals were bled to death, their sera were inactivated, ampouled, and stored in an icebox at 4° to 7° C. One cubic centimeter of this serum will yield about 15 c.c. of testing fluid after proper dilution and absorption. Before any testing fluid was ampouled, it was checked against several known control red blood cell suspensions. For example, anti-A test fluid was tested against OM, BN, AMN, and AN cells, and, of course, definite agglutination took place with the last two only, namely, AMN and AN. No preservative was added to the ampoules.

Anti-B.—Ten rabbits were given intravenous injections of BN red blood cell suspensions in a fashion similar to that described under "Anti-M."

At the end of the third course 4 rabbits remained alive and their four sera were examined in a manner analogous to that described for anti-A sera. One portion of the diluted anti-B sera was absorbed with pooled OM, OMN, AM, and AMN cells, thereby removing the species agglutinins and also any anti-A, anti-M, and anti-N agglutinins that might be present. The supernatant fluids, after absorption and centrifugation, were tested against known red blood cell suspensions of OMN, ON, AMN, and BMN blood. All four testing fluids reacted very weakly against BMN cells. The sera, therefore, from which these testing fluids were prepared were considered unsatisfactory for use. The unsuccessful result of this experiment conforms with the observation that it is far more difficult to prepare satisfactory anti-B immune sera than anti-A sera.¹⁰

As with the anti-A sera, the content of anti-N agglutinins was determined. The other portion of the inactivated and diluted anti-B serum was absorbed with OM, AM, and BM packed, pooled, and washed red blood cells. After absorption, the four fluids were tested against known AM, BM, ON, and OMN cell suspensions. The agglutination reactions against the N cells were weak and therefore the sera were not satisfactory for use.

DISCUSSION

In general, to prepare an immune anti-serum the following outline is advised:

1. Injection of specific antigen into sufficient number of rabbits.
2. Collection of small amount of serum from these animals for preliminary testing purposes.
3. Inactivation and dilution of these sera.
4. Absorption experiments until specific serum reacts with only its homologous antigen.
5. Titration.

Should the titer of anti-A, anti-B, or anti-M agglutinins reach 20 or higher, those rabbits are bled, their sera are inactivated and stored in an icebox as "Stock Serum." When, let us say, anti-M testing fluid is needed, one of these

ampoules of "anti-M Stock Serum" is diluted, absorbed, tested, and ampouled. With anti-N fluid, a titer of 10 or more is considered satisfactory.

As will be noted, a fairly large amount of blood is necessary both for the injections and especially for the absorptions. In a large hospital with its many steady employees and with its "Blood Bank," this seeming difficulty can be overcome.

An interesting observation is that in the present study we were far more successful in obtaining satisfactory anti-M sera than anti-N sera, which is contrary to the experience of most of the previous workers. This, as well as the variability in the response of the rabbits receiving AN blood (two forming good N antibodies but only weak anti-A; and the other two forming strong anti-A but practically no anti-N), serves to emphasize the importance of the individuality of the rabbit in relation to the results of the immunizations.

SUMMARY

1. Of 7 rabbits immunized with OM cells, 6 produced satisfactory anti-M serum.
2. Of 6 rabbits immunized with ON cells, 2 produced satisfactory anti-N serum.
3. Of 4 rabbits immunized with AN cells, 2 produced satisfactory anti-A serum and the other 2 produced satisfactory anti-N serum.
4. Of 4 rabbits immunized with BN cells, none produced a usable anti-serum.

I wish to take this opportunity to thank Dr. A. S. Wiener, Serologist to the Office of the Chief Medical Examiner of New York City, for his aid and many suggestions.

REFERENCES

1. Hooker, S. B., and Anderson, L. M.: Specific Antigenic Properties of Four Groups of Human Erythrocytes, *J. Immunol.* 6: 419, 1921.
2. Landsteiner, K.: Ueber Serumagglutinine, *München med. Wchnschr.* p. 1905, 1902.
3. von Dungern, E., and Hirschfeld, L.: Ueber gruppenspezifische Strukturen des Blutes, *Ztschr. f. Immunitätsforsch. u. exper. Therap.* 8: 526, 1911.
4. Landsteiner, K., and Levine, P.: A New Agglutinable Factor Differentiating Individual Human Bloods, *Proc. Soc. Exper. Biol. & Med.* 24: 600, 1927.
5. Landsteiner, K., and Levine, P.: Further Observations on Individual Differences of Human Blood, *Proc. Soc. Exper. Biol. & Med.* 24: 941, 1927.
6. Landsteiner, K., and Levine, P.: On Individual Differences in Human Blood, *J. Exper. Med.* 47: 757, 1928.
7. Wiener, A. S.: Blood Groups and Blood Transfusions, ed. 2, 1939, Springfield, Ill., Charles C. Thomas (a) p. 153; (b) Chapter 12.
8. Wiener, A. S., Zinsler, R., and Selkove, J.: The Agglutinogens M and N of Landsteiner and Levine, *J. Immunol.* 27: 431, 1934.
9. Davidsohn, I., and Rosenfeld, I.: The Preparation of Anti-M and Anti-N Testing Fluids, *Am. J. Clin. Path.* 9: 397, 1939.
10. Boyd, W. C.: Production and Preservation of Specific Anti-sera for Blood Group Factors, A, B, M, and N, *J. Immunol.* 37: 65, 1939.
11. Schiff, F.: Die Technik der Blutgruppenuntersuchung, Berlin, 1932.
12. Stuart, C. A., Sawin, P. B., Wheeler, K. M., and Battey, S.: Group Specific Agglutinins in Rabbit Serum for Human Cells. I. Normal Group-specific Agglutinins, *J. Immunol.* 31: 25, 1936.
13. Stuart, C. A., Sawin, P. B., Griffin, A. M., and Wheeler, K. M.: II. Immune Group Specific A Agglutinins, *J. Immunol.* 31: 31, 1936.
14. Battey, S., Stuart, C. A., and Wheeler, K. M.: IV. Immune Group-specific Beta Agglutinins, *J. Immunol.* 35: 75, 1938.
15. Wheeler, K. M., Sawin, P. B., and Stuart, C. A.: VI. Immune Specific M Agglutinins, *J. Immunol.* 37: 159, 1939.
16. Gettler, A. O., and Kramer, H. E.: Blood Grouping in Forensic Medicine, *J. Immunol.* 31: 321, 1936.

17. Boyd, W. C., and Boyd, L. G.: Blood Grouping in Forensic Medicine, *J. Immunol.* 33: 159, 1937.
18. Wiener, A. S.: Determination of Non-paternity by Means of Blood Groups, *Am. J. M. Sc.* 186: 257, 1933.
19. Wiener, A. S.: Medicolegal Applications of Blood Grouping, *Vida Nueva* 42: No. 2, 1938. English and Spanish.
20. Boyd, W. C., and Boyd, L. G.: New Data on Blood Groups and Other Inherited Factors in Europe and Egypt, *Am. J. Phys. Anthropol.* 23: 49, 1937.
21. Schaefer, G., and Wiener, A. S.: Limitations in the Use of Preserved Blood for Transfusions, *Quar. Bull., Sea View Hosp.* 5: 17, 1939.
22. Wiener, A. S., and Schaefer, G.: Limitations in the Use of Preserved Blood for Transfusions. A Study of the Fate of the Transfused Erythrocytes in the Patient's Circulation, *M. Clin. North America* 24: 705, 1940.

AN APPARATUS TO RECORD MOVEMENT OF THE EXTREMITIES*

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THERE is a need for some simple method of recording which enables one to analyze quantitatively the ability to perform coordinated movements of the extremities and to show any abnormalities which may be present. It is only by such means that it will be possible to demonstrate the degree of functional disability which is present in patients with injury of the nervous system and to assay adequately the value of treatment.

The apparatus described here was devised primarily to study the value of drug therapy in patients with the Parkinsonian syndrome. These patients show great diminution in the range and speed of voluntary movements, together with a rhythmical tremor of the extremities. These may be influenced by certain drugs, as has been described repeatedly in the literature. It has, however, been impossible hitherto to obtain accurate comparative information concerning the relative value of these substances and the dose which gives the most satisfactory results. It is also apparent that a method satisfactory for this purpose could be adapted to a study of other abnormalities of muscular control, such as those seen in patients with athetosis or the cerebellar syndrome.

Doshay (1938) described a method of determining the "graphic rigidity index" in patients with the Parkinsonian syndrome. This is essentially a means of calculating the frequency with which groups of muscles can be contracted or relaxed and the rate at which the movements tire. The effect of atropine on these factors was studied. The apparatus used was not discussed in detail. The principles involved were similar to those which we have developed independently.

DESCRIPTION OF APPARATUS

The apparatus shown in Fig. 1 works as a pulley system, recording the efforts of a patient to move a weight in a vertical plane by contractions of the muscles at the elbow or wrist. During the wrist movement the forearm is held fixed against the arm of a chair. A lever, which is connected to the weight by means of a thread passed through a pulley, writes upon a kymograph. Time is registered at second intervals.

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While one hand is raising and lowering the weight, the other is at rest holding a balloon. This is inflated lightly and connected by means of rubber tubing to a tambour, the arm of which writes on the kymograph. Any tremor in the fingers of the resting hand is recorded by this method.

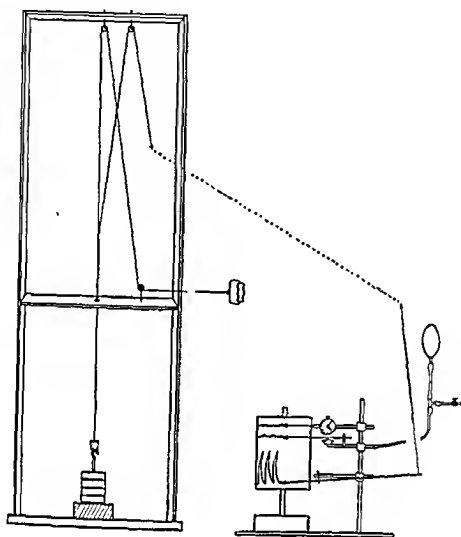


FIG. 1.

The patient is usually asked to raise and lower the weight a number of times as fast as possible, and then more slowly. The latter procedure is omitted if the maximum speed is greatly retarded. The result of maximal effort gives a good estimate of any rigidity which is present. The slow contraction, in particular, may show a superimposed tremor. However, in patients exhibiting the Parkinsonian syndrome the tremor is best portrayed in most cases by the balloon system from the resting hand. It is usually exacerbated during the time the patient is lifting the weight when his attention is withdrawn from this hand (Fig. 2) and when there is some emotional excitation.

Often the speed of contraction and relaxation in the individual with the Parkinsonian syndrome is greater when the weight which is lifted is increased from less than one pound to four or five pounds. Perhaps this is because the speed of the passive phase of the movement is increased and the interval between the active and passive phases is shortened.

This apparatus is not yet entirely satisfactory to record fast movements because of the rebound which is present in the apparatus. Consequently, it is difficult to demonstrate slight rigidity. It is hoped that this difficulty may be overcome by technical improvements.

DESCRIPTION OF RECORDS

For the purpose of illustration two records made from a patient with the Parkinsonian syndrome are shown in Figs. 2 and 3. Fig. 2 illustrates movements of the wrist, while Fig. 3 illustrates movements of the elbow. The upper tracing in both records indicates time in seconds, the middle one tremor in the resting hand and the lower one the actual muscle contraction. In the latter the upstroke indicates contraction and the downstroke relaxation.

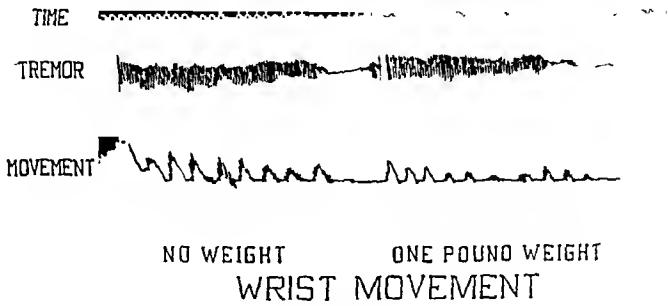


Fig. 2.

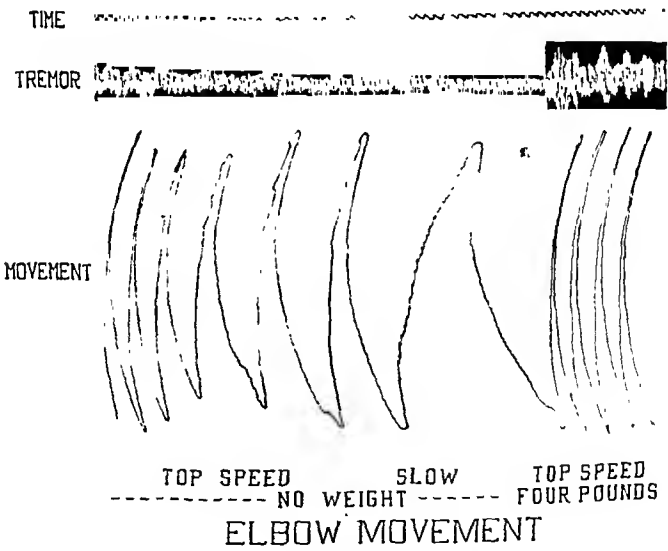


Fig. 3.

In Fig. 2 the rhythmical tremor is present in the resting hand throughout the period of wrist movement. The tremors also are superimposed upon the tracing of muscle contraction, showing graphically the well-known cogwheel phenomenon. The amplitude of the wrist contractions tends to decrease as the act is repeated; it is less when the weight is increased.

Fig. 3 illustrates the movements of elbow flexion and extension. The tremor is well shown during the period of active flexion when the movement is performed slowly. The tremor in the resting hand is increased when the patient is pulling a weight of four pounds; then it undergoes exaggeration during the active flexor movements.

SUMMARY

A method of graphically recording voluntary movements and tremor in patients with the Parkinsonian syndrome is described. The apparatus is inexpensive and gives reliable records. It has been utilized to test the effect of drugs upon the rigidity and tremor which these patients exhibit.

REFERENCE

Dashay, L. J.: Graphic Rigidity Index, *J. Mt. Sinai Hosp.* 5: 451, 1938.

A SIMPLE, CLEAN, CEDAR OIL BOTTLE*

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CEDAR oil bottles, as a rule fitted with glass, wooden, or metal applicators that dip into a pool of oil, are not satisfactory because it is difficult to control the amount of oil desired and the direction of the oil on the slide. As a result, the bottle, the hands, the microscope, and its stage may become smeared with cedar oil.



FIG. 1.

The bottle described herein offsets these difficulties. It is very simple to make. We have found it to be clean and very convenient.

An ordinary stain dropper bottle is used. The tip is drawn out to a fine point in the pilot light of a Bunsen burner. The end is cut off, leaving a very small opening at the end of the dropper. It is important to have the opening exactly of the right caliber. If it is too large, the oil will drop by gravity and drip into the bottle. If it is too small, too much pressure will be required to get the proper flow.

The hollow inner part of the dropper is filled with oil by means of a Pasteur pipette, and the rubber teat is applied. The bottle is now ready for use.

When needed, the oil is merely dropped on any part of the slide just like a stain, and the amount to be applied is regulated by the degree of pressure on the rubber teat.

The dropper is refilled whenever necessary.

Since the dropper and sides of the bottle are ground and fitted, there is no evaporation and hardening of the oil.

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HEMATOCRIT TUBE FILLING PIPETTE*

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THE instruments and methods used in filling and in cleaning the fine bore hematocrit tubes of the Wintrobe type, now employed extensively for determining the blood sedimentation rate and erythrocyte volume in patients with arthritis, tuberculosis, or other infections, have been open to criticism on several points. We have described a new and simple device for the rapid cleaning and drying of such tubes.¹ Here we shall offer a solution to the vexing situation of constant breakage of the slender glass pipettes now employed to fill this type of hematocrit tube. This breakage is costly in time, and often in material, to the clinics where many such blood studies are made.



Fig. 1.—Hematocrit tube filling pipette. Refer to text for explanation of lettering.

The filling pipette here described (Fig. 1) is relatively nonbreakable under more than average usage. The slender stem (A), which extends to the full depth of the tube to eliminate bubble formation, is made from a stainless lumbar puncture needle by the removal of the beveled pointed tip. The thick ground glass shank of the glass bulb (B) is fashioned to fit snugly into the adapter of the needle, thus making a leak-proof yet easily detachable joint, such as is used with the common syringe. The bulb is made of glass to permit visualization of its contents, and holds about 2 cm. of blood. A rubber bulb (C) is added to this to facilitate the exchange of the oxalated blood via the filling pipette.

This device is quickly cleaned by simply detaching the parts and attaching them to the suction tubing used for cleaning the ordinary blood pipettes and the hematocrit tube itself.

REFERENCE

1. Stirling, A. C.: Hematocrit Tube Cleaner, *J. LAB. & CLIN. MED.* 26: 393, 1940.
- This filling pipette is available either separately or in combination with the Wintrobe hematocrit tube and cleaner through the Will Corporation, Rochester, N. Y.

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THE DIAGNOSTIC VALUE OF THE COLLOIDAL CARBON FLOCCULATION TEST IN SPINAL FLUID*

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IN A PREVIOUS paper¹ we investigated the diagnostic value of the Takata-Ara reaction of the cerebrospinal fluid in neurosyphilis. The purpose of this paper is to prove the value of the colloidal carbon flocculation test in spinal fluid in cases of neurosyphilis and to compare the results with the Lange test and the Takata-Ara reaction.

Benedek and von Thurzó² introduced the so-called Kolloid-Schultz Index test for the differential diagnosis of diseases of the central nervous system. Other workers³ were unable to confirm their results.

Looney,⁴ and later Looney and Stratton,⁵ prepared a stable colloidal suspension of carbon by electrolysis between two carbon electrodes. Their method, interpretation, and results were very similar to the Lange test. They stressed the fact that the test is at least as sensitive as the Lange test and that the suspension is more stable.

Schube and Harms⁶ modified and simplified Looney's method. These workers, and later Deadman, Elliott, and Smith⁷ showed that in cases of positive Wassermann reaction in spinal fluid the colloidal carbon flocculation test in about 90 to 95 per cent of the cases was also positive. Various workers confirmed these results, especially Selesnick,⁸ who examined 478 cases. Of these, there were 55 cases of neurosyphilis, and in every case of a positive spinal Wassermann he found a positive colloidal carbon flocculation test. In over 400 cases where the spinal fluid was negative for syphilis there were only eight "false positive" reactions. In these "false positive" cases he found rapidly growing bacteria in the spinal fluid which he stated were the causes for the positive reactions.

METHOD

We used the method of Schube and Harms⁶ with the exception that we found that four tubes gave sufficiently accurate results. Into each of four Wassermann tubes are placed 1 c.c. of distilled water. One cubic centimeter of spinal fluid is placed in the first tube which is well agitated, and 1 c.c. of the mixture is transferred to the second tube, etc. One cubic centimeter of fluid is discarded from the fourth tube. In a fifth tube one cubic centimeter of distilled water is used as a control. Into each tube is now placed 0.1 c.c. of a 0.1 per cent oxalic acid solution and then 0.4 c.c. of a 1 per cent colloidal carbon suspension. The resulting mixture is well agitated. The colloidal carbon solu-

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tion we used was prepared as follows. One cubic centimeter of Carter's Black India Ink No. 358 is diluted with distilled water up to 100 c.c., and this is the reagent used.

Interpretation.—The flocculation test is considered positive when there is complete precipitation and the supernatant fluid is absolutely clear. If these conditions occur in any one of the four tubes, the reaction is considered positive. The results are read after twelve hours.

MATERIAL AND RESULTS

We examined 268 patients of whom there were 156 with general paresis. Of the 112 nonparetic patients 46 had dementia praecox, 39 had chronic alcoholism, 14 had cerebral arteriosclerosis, 6 had idiopathic epilepsy, and 7 were mentally deficient.

In every nonsyphilitic case the test was negative. In 141 cases of positive spinal Wassermann the colloidal carbon flocculation test was also positive. In the other 15 cases of positive Wassermann the colloidal carbon flocculation test was negative, or, in other words, the test was positive in 90.4 per cent of the cases of general paresis.

COMPARISON WITH OTHER TESTS

1. Gold sol test. In 44 cases with typical first zone curve (paretic curve) the colloidal carbon flocculation test was always positive. In 51 cases with a middle zone curve, the colloidal carbon flocculation test was positive in 49 cases. In 10 cases with an end zone curve, the colloidal carbon flocculation test was positive in 8 cases. In 47 treated cases with a negative Lange, but positive Wassermann, the colloidal carbon flocculation test was positive in 36 cases.

2. Takata-Ara test. We compared the results in 134 cases of general paresis. In 104 cases where the Takata-Ara test was positive, the colloidal carbon flocculation test was positive in 102 cases. In 31 cases of negative Takata-Ara reaction the colloidal carbon flocculation test was positive in 25 cases.

COMMENT

The colloidal carbon flocculation test appears to be more sensitive than the Lange and Takata-Ara tests. It is positive in about 90 per cent of cases where the spinal Wassermann is positive. The test continues to be positive in treated cases of general paresis, where the Ross-Jones test, cell count and Lange test are already normal. Our cases gave no "false positive" results. Because of the simplicity of the test, it can be used where ordinary laboratory facilities are not available.

SUMMARY

The colloidal carbon flocculation test was positive in 90.4 per cent of cases of general paresis with a positive spinal Wassermann.

The test is more sensitive than the gold sol and Takata-Ara tests.

REFERENCES

1. Szanto, Paul B., and Burack, Samuel: The Diagnostic Value of the Takata-Ara Reaction in the Cerebrospinal Fluid, *J. LAB. & CLIN. MED.* 26: 1079, 1940.
2. Benedek, L., and von Thurzó, E.: India Ink in Diagnosis of Nervous Diseases, *Ztschr. f. d. ges. Neurol. u. Psychiat.* 119: 69, 1929.

3. Sacristan, J. M., Salas, J., and Fanjul, L.: Diagnostic Value of India Ink Colloid Reaction, *Ztschr. f. d. ges. Neurol. u. Psychiat.* 123: 694, 1930.
4. Looney, J. M.: The Preparation and Use of Colloidal Carbon Solutions, *J. Biol. Chem.* 74: 63, 1927.
5. Looney, J. M., and Stratton, F. C.: The Preparation and Use of Colloidal Carbon Solutions, *J. LAB. & CLIN. MED.* 19: 69, 1929.
6. Schube, P. G., and Harms, H. E.: A Colloidal Carbon Flocculation Test in Spinal Fluid, *J. LAB. & CLIN. MED.* 18: 65, 1932.
7. Deadman, W. J., Elliott, F. J., and Smith, H.: The Examination of Cerebro-Spinal Fluids by Colloidal Carbon, *Am. J. Clin. Path.* 7: 246, 1937.
8. Selesnick, Sydney: The Colloidal Carbon Flocculation Test in Spinal Fluid for the Diagnosis of Neurosyphilis, *J. LAB. & CLIN. MED.* 23: 1068, 1938.

100 EAST JEFFERY STREET

AN AUTOMATIC BLOOD PRESSURE RECORDING APPARATUS*

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THERE is at present no device or principle that permits the continuous, simultaneous, and automatic recording of the systolic and diastolic pressures, the pulse pressure, and the pulse rate. Such a device would open a wide field of clinical and experimental investigation. It also has obvious practical values.

This communication describes such a basic principle and gives the description of an instrument that will furnish these data. The apparatus is designed to permit readings at fixed intervals over predetermined periods and under varying conditions, such as sleep, rest, and moderate activity. The results, furthermore, are entirely comparable with the readings obtained by the ordinary auscultatory method.

The principle is that of a fluctuating base line resembling a sine curve, which is projected onto a calibrated film or paper by means of a moving oscilloscope, galvanometer, or similar device. This fluctuating base line is produced by, and corresponds to, the changing pressures in a blood pressure cuff. The sensitive element within the oscilloscope (crystal, or a conductor in a magnetic field) or the galvanometer (conducting string) is then independently activated by sounds picked up microphonically at the antecubital space. This results in a deflection from the base line by each sound produced. These sounds are produced with each pulse beat in the pressure range between systole and diastole, and the points at which they appear or disappear represent the systolic and diastolic pressures. The pressure range between these two records the pulse pressure. The pulse rate is obtained by imposing a timer element on the record.

The apparatus is shown in Figs. 1 to 4. Fig. 5 is a representation of the graph obtained. The apparatus consists of a flat metal base plate (1) to which is rigidly attached an inverted U-shaped holder (2). The points of attachment

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are at (3) and (4). At the midpoint of the horizontal member or roof of this inverted U-shaped holder, there is a small socket (8). On the lower surface of this socket there is a smooth depression (9) to receive a pivot. Opposite this socket and rigidly attached to the base plate midway between the two legs of the inverted U there is another socket (10) which also contains a similar smooth depression (11) and into which another pivot fits.

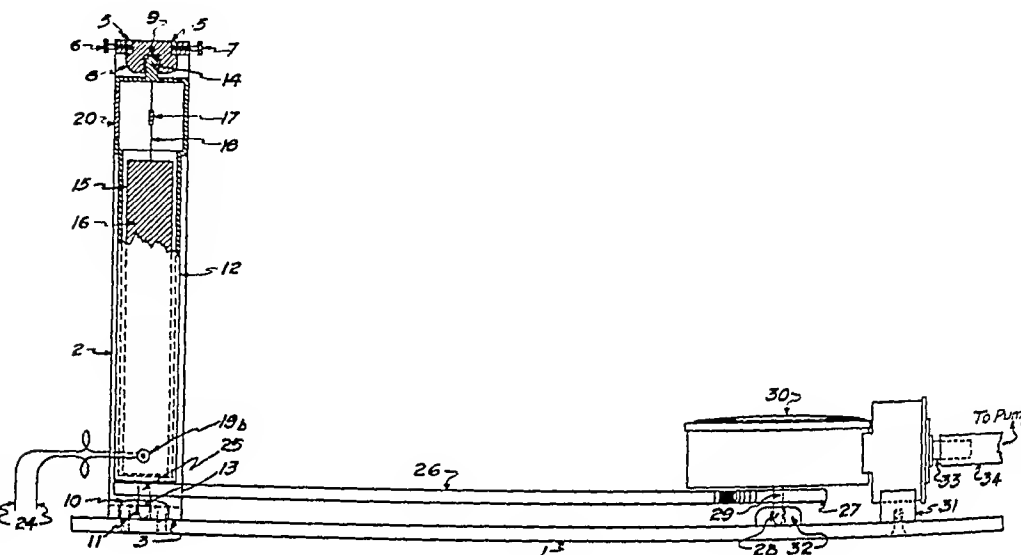


Fig. 1.

An oscillometer, otherwise known as an oscilloscope, is suspended between these two pivotal points so that it can rotate freely both clockwise and counter-clockwise. There are two pivots fixed to the oscillometer, one at its lower end (13) and the other at its upper end (14). These fit into the corresponding sockets (9 and 11).

The oscilloscope* shown in the drawings consists of a hermetically sealed chamber (15) in which there is a crystal bimorph element (16) fixed to its casing at the lower end, the upper end being free. To this free end a small mirror (17) is attached by means of a filament (18). This end is thus free to vibrate torsionally and independently, and with it the attached mirror. There are two contacts (19 a and b) leading from the bimorph element within the oscilloscope to which conducting wires are attached so that a current may be induced to flow through the element, causing it and the attached mirror to vibrate as described. The unit is filled with oil which serves as a damping agent and also protects the crystal against physical shocks. A window (20) in the head of the oscilloscope, sealed by a plano-convex lens affords access to the mirror and enables the light from the mirror to be brought to a focus without any additional optical parts.

A beam of light reflected from the mirror through the window moves with the movement of the mirror and is recorded on a recording camera. The source of light can be independent of the apparatus shown, or it may be fixed as

*The oscilloscope described is a modification of the Brush Piezo-Electric Oscilloscope Unit.

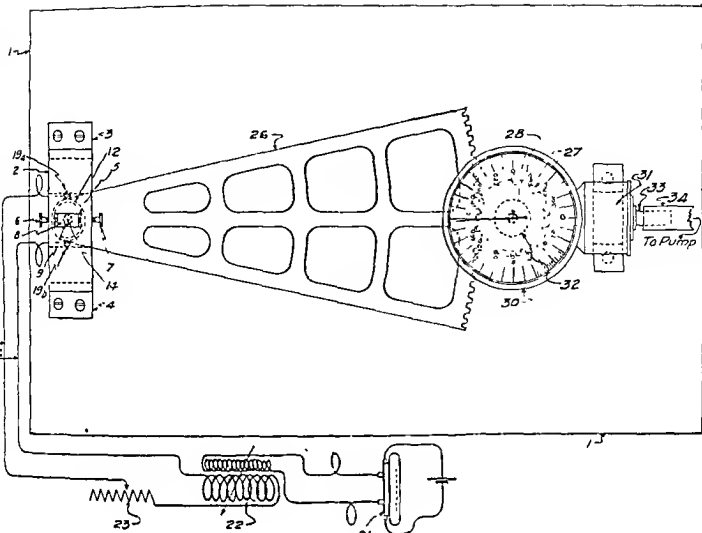


Fig. 2.

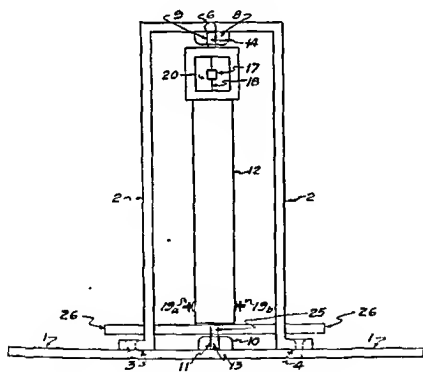


FIG. 3.

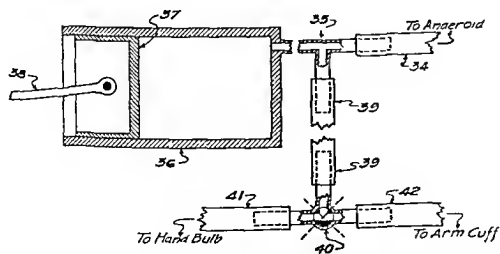


Fig. 4.

desired, either to the base plate, to the inverted U member, to the oscilloscope or to the upper pivot of the oscilloscope extended through the top of the inverted U. Its position depends on the desired placement of the camera which is controlled by factors of convenience and compactness.

The oscilloscope described in this communication and on the drawings is a crystal bimorph unit, but any other oscillometer, such as those commonly used in electrical practice which employ a conductor in a magnetic field, can serve the same purpose.

The current activating the oscilloscope element and mirror to move within its case comes from a microphone (21) which is appropriately electrified and is designed to be applied to the antecubital space of the arm below a blood pressure cuff. The current from this microphone, induced by the sound produced at the antecubital space, then passes through an appropriate apparatus (induction coil, resistance, etc.) to the binding posts of the oscilloscope, and as a sound is produced at the microphone the mirror within the oscilloscope twists and the beam of light reflected into the camera from the mirror records this movement.

Rigidly attached to the oscilloscope at (25) is a section of a gear (26). This gear meshes with a second gear (27), which is rigidly attached to the pivotal shaft (28) of an anaeroid (30) at point (29). The anaeroid is fixed through a saddle (31) to the base plate. The air entrance and outlet of the anaeroid is at (33), and to this is attached a tube (34) leading through a T to an air pump (36) or metal bellows. These can be adjusted for desired pressure ranges.

The other leg of the T (35) is attached through a tube (39) to a three-way valve (40). Through this three-way valve two other connections are made, one through a tube (41) to a hand bulb such as is used in the ordinary blood pressure apparatus and which incorporates a by-pass to the outside air, the other through a tube (42) to a blood pressure cuff which encircles the arm just above the point of application or attachment of the microphone at the antecubital space. The hand bulb is used for purposes of adjustment. During the automatic operation of the instrument, it is closed off at the three-way valve (40) and the arm cuff is open to the pump.

OPERATION

The apparatus operates as follows: The motor, geared down to a slow speed, operates the eccentric to which the piston shaft (38) is attached. This causes a slow reciprocating action of the piston head (37) in the pump cylinder (36) and results in the reciprocal movement of air into and out of the anaeroid (30) and the arm cuff. With this rising and falling of the air pressure there is a corresponding clockwise and counter-clockwise movement of the pivotal shaft (29) of the anaeroid (30). As this takes place the gear (27), rigidly attached to this pivotal shaft, moves likewise. The gear (27) meshes with the oscilloscope gear segment (26), causing it to rotate backward and forward. This gear segment (26), being rigidly attached to the oscilloscope, causes that unit to rotate both counterclockwise and clockwise.

A beam of light is reflected from the oscilloscope mirror (17) onto the moving sensitized film or paper of the camera, and a line is obtained resembling a sine curve which is smooth and represents the base line upon which the blood pressure readings are to be recorded. This base line is calibrated to run between any desired blood pressure levels by means of the displacement permitted the pump cylinder head within the pump casing. The upper limit of its excursion is adjusted and fixed through the motor-driven eccentric, the lower limit through the hand bulb. It is, of course, preferable that this lower limit be at a pressure of zero millimeters of mercury for the convenience and comfort of the patient.

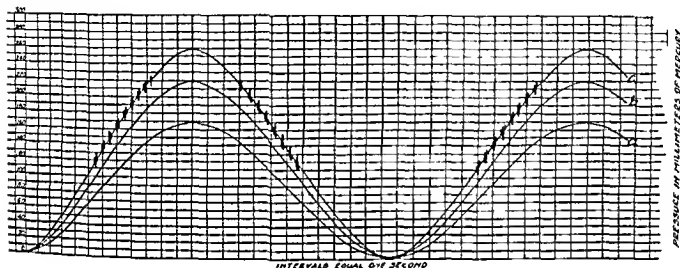


Fig. 5.

This smooth basic curve is shown in Fig. 5a. If it is desired to take readings at a higher blood pressure level and over a larger range, the eccentricity of the driving motor is increased and the basic curve will be as shown in Fig. 5b.

As the apparatus is being alternately inflated and deflated by the pump and the basic line is being drawn, the pressure in the arm cuff rises and falls in corresponding fashion. As the pressure rises from zero to the diastolic pressure, no sound is produced in the antecubital space up to the point of diastole where the sound appears because the artery is now being compressed. This sound is detected by the microphone (21) fixed over the artery in this space. As the pressure continues to rise, these sounds persist with each heart beat until the pressure rises to the point of systole when the sounds disappear because the artery is now completely occluded. As the pressure falls the same events take place, except that the sequence is now reversed.

The sounds produced in the antecubital space and picked up by the microphone (21) give rise to electrical impulses which are transmitted to the crystal (16) (or field loop) of the oscilloscope (12), causing it to distort or twist and simultaneously to twist the attached mirror (17) through the suspension (18). This distortion or twist causes a deviation from the smooth base line described and produces irregularities in this line which represent at their beginning and ending the points of systole and diastole. Such a curve is represented in Fig. 5c.

Pulse rates are obtained by means of a timer recording on the film.

The apparatus is automatically controlled by means of a clock mechanism to take records at various predetermined intervals and over any desired periods. The automatic control of the instrument, which is not new and therefore not shown, consists of a timing device which regulates the stopping and starting at fixed points and permits the instrument to run for fixed periods. It simultaneously switches on and off the pump motor, the camera motor, the source of light, the camera shutter, the timer, and the microphone. The "make and break" is planned to take place at the lowest pressure phase so that there may be no pressure on the arm during the intervals when readings are not being made.

SUMMARY

A blood pressure recording device is described which functions automatically and which can record both systolic and diastolic pressure readings. These readings can be taken in the absence of a physician or operator, permitting blood pressure recording during sleep, rest, and various phases of activity. The records obtained are entirely comparable with the readings obtained by the ordinary auscultatory method. The instrument also records the pulse pressure and the pulse rate.

The instrument functions as a complete unit for the purpose described. It is intended to employ it further as part of a cardiodynamometer to measure relative cardiac efficiency.

Addendum.—During the development of this instrument other devices were designed which employ the same basic principle, that of a sound-sensitive element moving in synchrony with an induced pressure. One such design utilizes a galvanometric string oscillating in a split magnetic field. Another employs a fixed galvanometer string and an oscillating camera. A third employs a mirror that moves torsionally with pressure change and reflects the shadow of a fixed galvanometer string into a fixed camera.

A MANUALLY OPERATED ERGOMETER*

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FOR a number of years it has been our custom to use a bicycle ergometer of special design in certain experiments in which the respiratory exchange was measured during muscular exercise. With certain patients difficulties were encountered, which led to the use of an ergometer which could be operated by hand while the patient was comfortably seated in a chair. In this ergometer, the work was measured by recording the output of an electrical generator. The principle has been employed by Kelso and Hellebrandt.¹

The construction of the ergometer is evident from Fig. 1. The sprocket tooth ratios were selected to drive the motor at approximately its rated speed with convenient cranking rates. The electrical circuit is diagrammed in Fig. 2. The motor generator acts as a brake, and the patient overcomes the resistances, both mechanical and electrical, and produces electrical power which is dissipated by the load resistances as heat. An easily readable voltmeter is placed before the patient, who maintains his output constant by watching it.

The method of calibration is a familiar one in electrical engineering practice:

1. Output of the subject = input to the ergometer.

2. Input to the ergometer = armature generated power plus power losses.

$$3. P = P_a + P_s \quad (1)$$

where P = output of subject in watts
 P_a = armature generated power
 P_s = power losses

$$4. P_a = I_a \times E_g \quad (2)$$

where I_a = armature current
 E_g = generated voltage

$$5. I_a = \frac{E_t}{R_l} \quad (3)$$

where E_t = terminal voltage
 R_l = resistance of load resistance

$$6. E_g = E_t + B_d + (I_a \times R_a) \quad (4)$$

where B_d = brush drop
 R_a = resistance of armature

$$7. P_a = \frac{E_t^2}{R_l} + \frac{E_t \times B_d}{R_l} + \frac{E_t^2 R_a}{R_l^2} \quad (5)$$

by substituting (3) and (4) in (2).

8. The ergometer was cranked by hand with selected field currents at arbitrarily chosen terminal voltages, and values for P_a at these values for E_t were calculated from (5).

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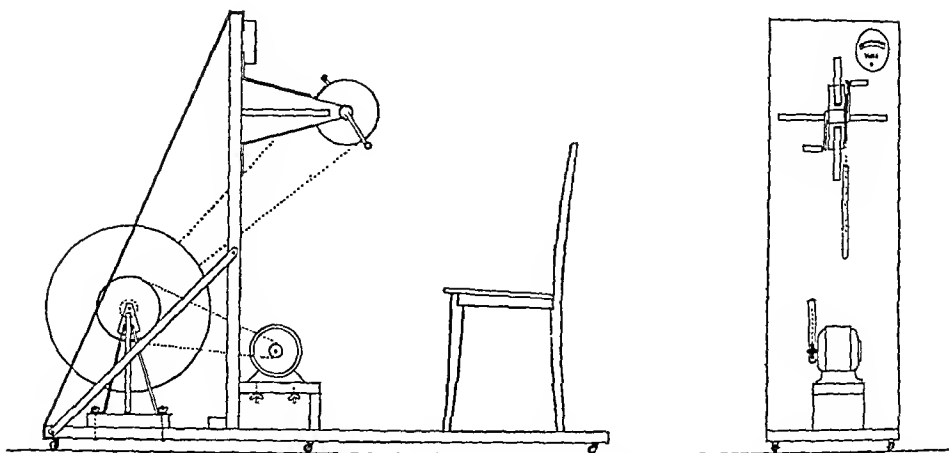


Fig. 1.—Construction of ergometer. The generator is a $\frac{1}{12}$ th horse power D.C. shunt wound motor.

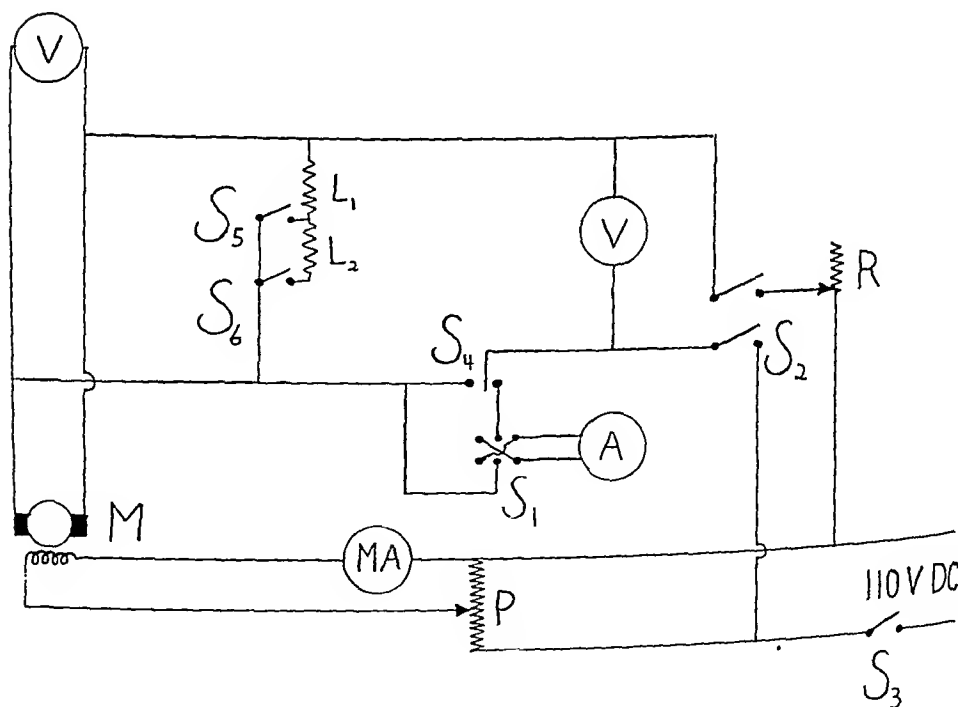


Fig. 2.—Electrical circuit. The field of the motor generator (M) is separately excited. The potentiometer (P) adjusts the field current to any desired value measured by the milliammeter (MA). The armature generated power is dissipated by the load resistances (L_1 and L_2) as heat. The voltmeters (V) are one for the patient and one for the observer. Power may be supplied to the armature for calibration purposes by the switch (S_2) and controlled by the resistance (R). The ammeter (A) and the ammeter reversing switch (S_1) are for the purposes of calibration also and may be thrown in and out of the circuit by a switch (S_1). A main power switch (S_3) is provided.

9. Power losses are of two sorts: "copper losses" and "stray power losses." When the ergometer was run motor action, the electrical input overcame these losses:

$$(E_a \times I_a) = P_c + P_s \quad (6)$$

where E_a = input voltage (effective armature voltage)

I_a = input amperage (armature current)

$E_a \times I_a$ = input power

P_c = copper losses

P_s = stray power losses

$$10. \text{Hence } E_a \times I_a = P_s + I_a^2 R_a + B_d I_a \quad (7)$$

where $I_a^2 R_a$ = armature copper loss

$B_d I_a$ = brush drop loss

$$11. \text{Now } E_a = E_t - B_d \quad (8)$$

$$12. \text{Hence } P_s = (E_t - B_d) I_a - I_a^2 R_a - B_d I_a \quad (9)$$

from (7) and (8)

where $P_c = I_a^2 R_a + B_d I_a$

13. The ergometer was run motor action and stray power losses for various r.p.m. were calculated from observed load terminal voltages (E_l) and armature current for several empirically chosen values of field current by (9).

14. The ergometer was operated with only a voltmeter in the armature circuit and no load terminal voltages for the r.p.m. used above were observed at the same field currents used in 13.

15. The voltage at the terminals at load is related to no load terminal voltages by the following:

$$E_l = E_{nl} - B_d - I_a R_a \quad (10)$$

where E_l = load terminal voltages

E_{nl} = no load terminal voltages

16. Substituting (3) in (10)

$$E_l = \frac{E_{nl} - B_d}{1 + R_a/R_l} \quad (11)$$

17. For any value of E_l a value of E_{nl} can be found and hence an r.p.m. for given field currents (steps 16 and 14).

18. By adding a value for armature generated power (from step 7) to the stray power loss corresponding to that terminal voltage (from 17), a total power is found which represents the input of the patient to the ergometer (1).

The power in watts was converted to kilogram meters per minute; from this a graph was prepared showing kilogram meters per minute versus load terminal voltage for the field currents and load resistances used (Fig. 3). The higher the field current, the lower the cranking rate is for the same amount of work. In use, the cranking rate and desired working rate are translated into load terminal voltage and field current. The operator brings the ergometer to speed and indicates to the patient the desired voltage on the voltmeter before him. He is instructed to take over the cranking at a given command. An observer records the terminal voltage every thirty seconds during the exercise period, and maintains the field current constant manually when necessary. At the end of the exercise period the patient releases the cranks and allows them to spin freely.

Table I shows four experiments comparing a normal person and a patient with cardiac disease on the hand and bicycle ergometers. Strict comparison is not possible because the amount of work is different, but two facts are apparent: The mechanical efficiency is greater with the bicycle ergometer, and exercise with the arms appears to augment the ventilation more than exercise with the legs.

TABLE I

COMPARISON OF TWO PATIENTS ON THE HAND AND ON THE BICYCLE ERGOMETER

SUBJECT	N.K. (NORMAL)		H.K. (CARDIAC)	
	Hand	Bicycle	Hand	Bicycle
Ergometer				
Time, minutes	3	3	3	5
Work, kilogram meters/minute	250	432	260	315
Work, calories	1.758	3.036	1.827	3.695
Excess heat production, calories	10.454	16.907	14.974	24.776
Net efficiency per cent	16.82	17.96	12.20	14.91
Ventilation/minute exercise	17.04	19.13	26.1	26.0
Total oxygen debt	0.520	1.127	1.688	2.356

DISCUSSION

No method of calibrating a motor is free from criticism. The method reviewed here is standard engineering practice and is fully discussed in textbooks on this subject. The generator used in the apparatus of Kelso and Hellebrandt was an automobile starting motor. It had advantages over the one

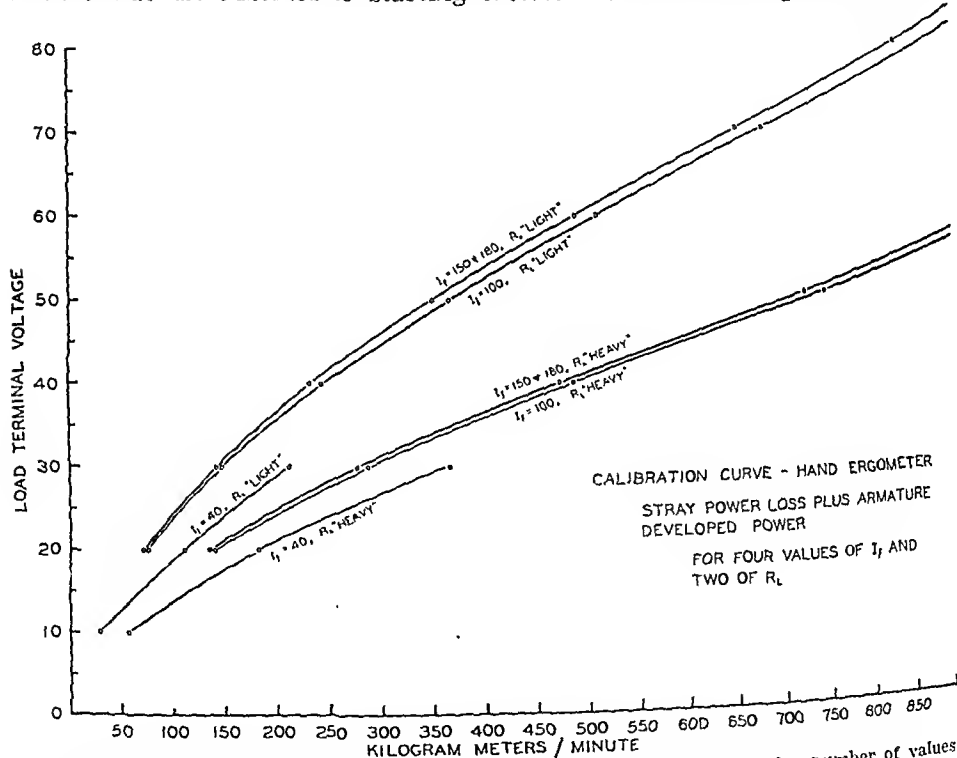


Fig. 3.—Calibration curve. At a selected value of work, any one of a number of values may be chosen, depending on the cranking rate suitable for the patient. The lower the field current, the greater the cranking rate for the same amount of work.

used in this ergometer. A family of curves was obtained in the calibration rather than a single curve for all values of field current as was the case with their machine. The advantage of the single curve is that the field current may be varied to change the cranking rate, yet the working rate will not vary as long as the terminal voltage is held at the same value.

It is advantageous to incorporate the motor power circuit permanently in the apparatus, as this makes it possible to check the calibration at any time in a few minutes.

SUMMARY

Certain objections to the use of the bicycle were overcome by construction of a manually operated ergometer. The motor-generator braking principle was used. The method of calibrating this apparatus is briefly reviewed. Comparison with the bicycle ergometer showed that the mechanical efficiency of the arms is less than that of the legs, and that exercise with the arms augments ventilation more than exercise with the legs.

REFERENCE

1. Kelso, L. E. A., and Hellebrandt, Frances A.: The Recording Electrodynamie Brake Bicycle Ergometer, *J. LAB. & CLIN. MED.* 19: 1105, 1934.

A TECHNIQUE FOR THE ARTIFICIAL INSEMINATION OF THE WHITE RAT*

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ARTIFICIAL insemination of laboratory mammals is of increasing importance in studies of the physiology of ova and spermatozoa. In the rabbit,^{1, 2} guinea pig,³ and dog,^{4, 5} artificial insemination may be successfully carried out by merely depositing a sperm suspension in the vagina. In the rat, on the other hand, numerous attempts to impregnate females in heat by depositing sperm suspensions in the vagina alone resulted in complete failures.

Tochem⁶ artificially inseminated rats by operating and injecting sperm suspensions directly through the uterine walls. Fragmentary reports relating to the artificial insemination of female rats and mice⁷ may be found in the literature, no one of them completely satisfactory in the life of recent developments.

The technique here described has been used successfully in the artificial insemination of over 500 female rats during a study of the fertilizing capacity of old ova.

As a basis for the proper time for insemination we have depended upon the behavioral responses or sexual receptivity as elicited in the female rat during heat.

When the region of the iliac crest of a female rat in heat is manually stroked, she assumes the characteristic posture of lordosis. This posture is characterized by an extension of the limbs, arching of the back and a raising of the pudendal area and tail. The onset of heat can be accurately determined by this response. The relationship between the onset of heat and ovulation is sufficiently definite that the latter may be said to occur approximately ten hours after the beginning of heat.⁸⁻¹¹

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Since the animals which were to be inseminated were not allowed to copulate, the cervix uteri of each animal was stimulated during heat by a strong faradic current, according to the technique of Greep and Hisaw,¹² and Astwood.¹³

When a female rat is to be inseminated, a normal adult male is killed by grasping it by the tail and striking its entire body sharply against a solid object. Any other method for killing usually results in struggling, urination, and ejaculation, with the resultant loss of sperm. This loss can be entirely avoided after a few trials in the proper method for killing the male.

The male is immediately opened by a ventral incision. The bladder is retracted posteriorly by a Kelly clamp, and the cauda epididymides and vasa deferentia are removed with a fine pair of scissors. Fat tissue adhering to the cauda epididymides is dissected away.

Contrary to the usual method of macerating the epididymides and vasa deferentia in Loeke's solution, and subsequently drawing off the sperm suspension from the debris, a technique has been devised by which a pure sperm suspension is obtained. This is of importance since the suspension must be drawn into a relatively fine hypodermic needle for insemination. It also eliminates the possibility of contamination by the presence of gross amounts of cellular debris.

The cauda epididymis is held between the fingers, and the cut end of the vas deferens is put into a small dish which is to receive the sperm. Using a medium point forceps, the epididymis is now massaged with increasing pressure toward the vas deferens. If this is done properly, ribbons of spermatozoa will be seen to exude from the distal end of the vas deferens. When sufficient spermatozoa have been removed from the epididymis, the massaging is continued along the vas deferens so as to force the remaining spermatozoa from it. Both epididymides are treated in a similar manner, the result being a viscid mass of densely packed spermatozoa. To this sperm mass is added 0.2 c.c. of Loeke's solution, which has been kept at 12° C. in a refrigerator. The suspension is then drawn into a tuberculin syringe to which has been attached a No. 21 hypodermic needle. The point of the needle has been ground down so as to present a blunt, smooth surface which will not injure the cervical canals. Care should be taken to have a thick, viscid suspension of spermatozoa for each insemination.

Just prior to insemination the female is lightly anesthetized with ether. During insemination the anesthetized animal is held by the base of the tail, with its back resting on the edge of an ordinary laboratory ring stand. An ear speculum of 5 mm. diameter at its smallest bore is placed deep into the vagina. By the use of a physician's reflecting mirror, the cervix may be illuminated and observed directly. The hypodermic needle is inserted into each cervical canal, and one-half of the sperm suspension is injected into each horn.

In order to be certain that the sperm suspension will remain in the uterine horns after insemination and not flow back into the vagina, especially if animals are inseminated near or after the end of heat, a plug is made in the following manner: A seminal vesicle with its associated coagulating gland is cut near its base and quickly brought to a flat, glass surface. The secretion is expressed from the seminal vesicle by stroking the gland with a probe. The secretion of the

coagulating gland must be expressed separately from that of the seminal vesicle, because if mixing of the two secretions occurs, coagulation immediately takes place. After the female has been inseminated, and while she is still under anesthesia, a small cotton plug is dipped, first, into the secretion of the seminal vesicle, then into the secretion of the coagulating gland. The plug is immediately transferred to the vagina through an ear speculum and pressed against the cervix. If done properly, the plug will simulate the vaginal plug formed during normal copulation and will aid in the retention of the spermatozoa in the uterine horns.

The percentage of impregnations from animals artificially inseminated during heat by this technique is as high as in animals which have been allowed to copulate normally. By use of this method, inseminations may be made at any time of the reproductive cycle when the female would otherwise not copulate.

REFERENCES

1. Heape, W.: The Artificial Insemination of Mammals and Subsequent Possible Fertilization or Impregnation of Their Ova, Roy. Soc. London 61: 52, 1897.
2. Hammond, J., and Asdell, S. N.: The Vitality of the Spermatozoa in the Male and Female Reproductive Tracts, Brit. J. Exper. Biol. 4: 155, 1926.
3. Blandau, R. J., and Young, W. C.: The Effects of Delayed Fertilization on the Development of the Guinea Pig Ovum, Am. J. Anat. 64: 303, 1930.
4. Spallanzani: Expériences pour Servir à l'Histoire de la Génération des Animaux et des Plantes, Genève, 1786.
5. Hermansson, K. A.: Kunstgjord Befruktning of Tik, Svenska Vet. Tidskr. 39: 382, 1934.
6. Yochem, D. E.: Spermatozoon Life in the Female Reproductive Tract of the Guinea Pig and Rat, Biol. Bull. 56: 274, 1929.
7. Merton, H.: Studies on Reproduction in the Albino Mouse. III. The Duration of Life of Spermatozoa in the Female Reproductive Tract, Proc. Roy. Soc. Edinburgh 59: 207, 1939.
8. Blandau, R. J., Boling, J. L., and Young, W. C.: The Normal Reproductive Cycle of the Female Rat as Determined by Means of the Copulatory Response, Anat. Rec. 75: 74, 1939.
9. Boling, J. L., Blandau, R. J., and Young, W. C.: The Time of Ovulation in the Female Rat in Relation to the Beginning of Heat, Anat. Rec. 75: 75, 1939.
10. Blandau, R. J., Boling, J. L., and Young, W. C.: The Length of Heat in the Albino Rat as Determined by the Copulatory Response, Anat. Rec. In press.
11. Boling, J. L., Blandau, R. J., Soderwall, A. L., and Young, W. C.: Growth of the Graafian Follicle and the Time of Ovulation in the Albino Rat, Anat. Rec. 79: 313, 1941.
12. Greep, R. O., and Hisaw, F. L.: Pseudopregnancies From Electrical Stimulation of the Cervix in the Diestrus, Proc. Soc. Exper. Biol. & Med. 39: 359, 1938.
13. Astwood, E. B.: An Assay Method for Progesterone Based Upon the Decidual Reaction in the Rat, J. Endocrinol. 1: 49, 1939.

A STUDY OF THE BLOOD CELLS OF NORMAL GUINEA PIGS*

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WAKE FOREST, N. C.

PREPARATORY to an experiment in which we were very much interested, it became necessary to know the normal blood cell picture of guinea pigs. This information was necessary before we could recognize any abnormal change that might occur in the blood during the course of the experiment. We wrote to two or three publishing companies for books or references describing the anatomy and physiology of these animals, but without any success. We were, of course, interested primarily in the cellular constituency of the blood. In the search for such information we found one author¹ who names with percentages the normal cellular elements of the blood of these animals, along with several other laboratory animals. Not being content, we set out to make daily blood examinations of a group of normal guinea pigs in order to establish an average normal. Our results are as shown below.

PROCEDURE

We obtained² nine normal full-grown guinea pigs (ages unknown). They were held under quarantine for several days to rule out the presence of any disease and to accustom them to new surroundings. They were kept in clean cages in a well-lighted and well-ventilated room, and fed a full-balanced diet. A part of the diet consisted Purina complete rabbit chow. This was reinforced with lettuce, cabbage, carrots, etc.

Examination of the blood was made daily for eighteen to twenty-one days. Blood was collected from the margin of the ears by making a small skin incision which allowed free bleeding. Each examination consisted of a red and white blood cell count and a differential. We did not do any hemoglobin determinations. Counts and smears were done in the usual way. Calculations which were used to arrive at an average normal were as follows: An average of the eighteen to twenty-one daily examinations for each animal was taken. Then from these averages the average for the nine guinea pigs was obtained.

RESULTS

Our study revealed that there was a rather wide variation in the total white and differential counts in some animals from day to day. However, there were not a great many counts that were conspicuously far away from the predominating range. The daily count of the red corpuscles, on the other hand, showed little variation and stayed within a rather narrow range. As to the individual white cells, there was moderate variation from day to day, and in a few instances a marked variation from the previous day. In these instances of marked

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variation, the succeeding counts did not show a return to the previous count, but continued to fluctuate. We noted nothing of any unusual size or shape of the cells.

Our conclusions were that the neutrophiles showed a range of from zero to 76 per cent, with an average of 31.1 per cent; the lymphocytes a range of from 20 to 97 per cent, with an average of 63.1 per cent; the monocytes ranged from zero to 10 per cent, with an average of 1.8 per cent; the eosinophiles ranged from zero to 14 per cent, with an average of 3.5 per cent; and the basophiles ranged from zero to 2 per cent, with an average of 0.19 per cent. The results of this study are summarized in Table I.

TABLE I*

ANIMAL NUMBER	R.B.C.	W.B.C.	NEUTRO- PHILES (PER CENT)	LYMPHO- CYTES (PER CENT)	MONO- CYTES (RANGE AND PER CENT)	EOSINO- PHILES (RANGE AND PER CENT)	BASO- PHILES (RANGE AND PER CENT)
1	5,228,095	20,345	28.6	63.7	0 to 10 2.4	0 to 13 5.0	0 to 2 0.2
2	5,044,286	14,214	31.4	65.5	0 to 8 1.8	0 to 4 1.0	0 to 2 0.1
3	5,033,805	13,031	32.9	61.2	0 to 3 0.8	0 to 11 4.7	0 to 2 0.2
4	4,789,762	17,267	37.0	59.8	0 to 6 2.3	0 to 2 0.5	0 to 2 0.2
5	4,941,429	13,227	18.0	79.6	0 to 6 1.5	0 to 3 0.7	0 0
6	5,114,762	23,750	33.7	60.3	0 to 6 1.6	0 to 8 3.9	0 to 2 0.3
7	5,257,619	17,386	30.9	60.1	0 to 8 1.9	0 to 14 6.8	0 to 2 0.2
8	5,095,733	17,294	36.1	57.7	0 to 7 2.6	0 to 12 3.2	0 to 2 0.3
9	5,079,231	20,672	31.6	60.7	0 to 5 1.6	0 to 12 6.0	0 to 1 0.04
Average†	5,063,970	17,365	31.1	63.1	1.8%	3.5%	0.19%

*These figures are averages of the twenty-one daily counts of each animal

†The bottom horizontal line of figures is the average count of the nine animals.

SUMMARY

1. Blood examinations were made daily of nine healthy guinea pigs for from eighteen to twenty-one days.
2. The animals were fed a full-balanced diet during the period of investigation.
3. Rather wide variation in the white blood cell count was noted from day to day. Moderate to marked variation was seen in the different types of white blood cells. The red blood cell count remained rather fixed within a narrow range.
4. Normal averages found were: red blood cells 5,063,970, white blood cells 17,365, neutrophiles 31.1 per cent, lymphocytes 63.1 per cent, monocytes 1.8 per cent, eosinophiles 3.5 per cent, and basophiles 0.19 per cent.

REFERENCES

1. Kracke, R. R., and Garver, H. E.: Diseases of the Blood and Atlas of Hematology, Philadelphia, J. B. Lippincott Co.
2. Kauffman, E. R., Norristown, Pa.

CHEMICAL

PROTHROMBIN ESTIMATION USING RUSSELL VIPER VENOM*

I. SIMPLE MODIFICATION OF QUICK'S METHOD

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THE determination of the prothrombin coagulation time in clinical practice is assuming increasing importance as knowledge of vitamin K deficiencies accumulates. The prothrombin estimation tests, in order to be of greatest service to the clinician, must be easy to perform, and their interpretation must be simple.

The tests for determining prothrombin time in general use (Quick, 1938; Smith and others, 1939; Kato and Poncher, 1940) employ thromboplastin prepared from tissue extract. Such extracts vary in potency and readily deteriorate, so that it is necessary frequently to check them against extracts of known potency. These factors lessen the efficiency and accuracy of the test and make it more difficult for the smaller clinical pathologic laboratories to use it to the fullest advantage.

In order to simplify the prothrombin estimation test, methods that do not employ tissue extracts have been developed, such as the modified Howell's method of prothrombin (Cheney), in which oxalated plasma is added to four tubes containing increasing amounts of calcium chloride (0.4 to 2 mg.), the shortest time of clot formation being taken as the coagulation time of the plasma.

A simple modification of Quick's method has been reported by Fullerton, who used Russell viper venom instead of tissue extract. Russell viper venom possesses the following advantages: (1) it is constant in potency, (2) it is in a clear solution, and (3) it permits more easily the observation of the end point of the formation of a fibrin web during the performance of the test than does the use of a milky tissue extract.

The following study was carried out because of these apparent advantages and in order to compare the prothrombin coagulation times on the blood of the same patients using the two methods.

METHODS

Blood of normal individuals, of patients in a general hospital population, and of patients attending outpatient clinics was used.

Four and a half cubic centimeters of venous blood were mixed with 10 mg. of potassium oxalate, centrifuged, and the oxalated plasma was obtained.

*From the Department of Pathology, St. Agnes Hospital, White Plains.
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Two tests were performed on each sample of plasma:

(1) Quick's (1938) method of prothrombin coagulation:

0.1 c.c. of oxalated plasma

0.1 c.c. of thromboplastin solution (rabbit brain)*

0.1 c.c. of calcium chloride solution

The time as determined with a stop watch from the addition of the calcium to the formation of a fibrin web was considered to be the prothrombin coagulation time.

Comparison of Prothrombin Coagulation Time between Quick's Method and Modified Quick's Method Using Russell's Viper Venom

□ QUICK'S METHOD ■ MODIFIED QUICK'S METHOD

N-Denotes Essentially Healthy Individuals

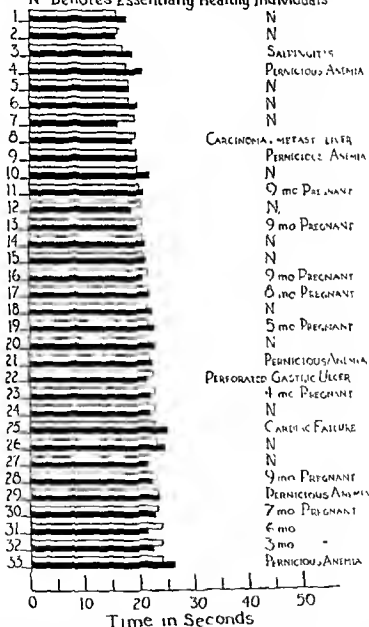


Fig. 1.—Prothrombin estimation using Russell viper venom and tissue extract.

(2) Modified Quick's method with Russell viper venom:

0.2 c.c. of oxalated plasma

0.2 c.c. of Russell viper venom (1:10,000) solution†

0.2 c.c. of calcium chloride solution (1.11 per cent calcium chloride)

The time from addition of the calcium chloride to the formation of a fibrin web was taken as the prothrombin coagulation time.

*Three different batches of thromboplastin were used in order to lessen the margin of error.

†"Sclpven" brand Russell viper venom for this study was supplied by Burroughs Wellcome & Co., New York, in vials of 0.1 mg. and 0.5 mg., with 1 c.c. and 5 c.c. of diluent, respectively, thus making a 1:10,000 solution when diluted.

RESULTS OF STUDY

Using two methods, prothrombin coagulation tests were performed on blood from 71 individuals. The results in graphic form with the diagnoses are given in Figs. 1 and 2. The prothrombin coagulation time, with Quick's method averaged 25.0 seconds, while that of the modified test with Russell viper venom averaged 24.7 seconds.

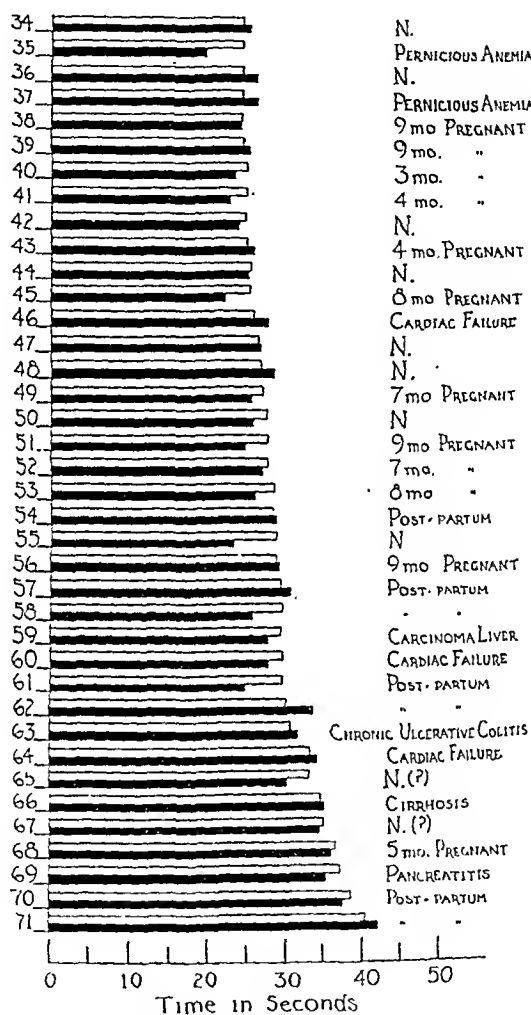


Fig. 2.—Prothrombin estimation using Russell viper venom and tissue extract.

The greatest variation between the two tests was found in the plasma of an essentially normal individual; by Quick's method the prothrombin time was 5.5 seconds longer than by the modified test with venom. In only six cases was the variation between the two tests more than three seconds.

Statistical evaluation (using the "t" formula of Fisher and Yates) of the results of the two tests gives a value of 1.638, which indicates that the time difference between the two tests is not significant. The prothrombin coagulation times, as determined by these two methods, differed so slightly that for all practical purposes the results obtained may be assumed to be identical.

For the purpose of this study it was considered that prothrombin time over thirty seconds was abnormal. In cases with a prothrombin time of less than 30 seconds it was found that when Russell viper venom was used, the average time of appearance of the fibrin web was 22.9 seconds. This figure closely checks with the average time of 21.7 seconds, as reported by Fullerton.

DISCUSSION

A modified Quick's method, using Russell viper venom instead of tissue extract, has been compared with the regularly used Quick's method for determination of prothrombin coagulation time. Comparison of tissue extract and Russell viper venom is shown in Table I.

TABLE I

	TISSUE EXTRACT	RUSSELL VIPER VENOM
Preparation	Usually prepared from brain tissue (rabbit, etc.) by laboratory in which it is to be used	Dried, sterilized, and packed in sealed vials
Standardization	Must be compared with extracts of known potency or by testing on plasma of known normal prothrombin content. Difficult to obtain extracts of constant potency	Standardized by comparing with a sample of known potency on fowl and horse plasma*
Deterioration	Gradually loses potency	Relatively stable in dry form; in solution it is stable for one month when kept in refrigerator and well-stoppered
End point of test; formation of fibrin web	Makes it difficult to determine because milky appearance of tissue extract obscures early recognition of fibrin web	Clear solution enables determination of fibrin web to be seen easily

*Information supplied by manufacturer.

Included in the study were seven post-partum patients within ten days of delivery. Their average prothrombin coagulation time was thirty-two seconds, which may be considered a borderline prothrombin deficiency. This may have been due to one or both of two factors, namely, loss of blood at time of parturition or a low-grade vitamin K deficiency. It is worthy of note that every one of the post-partum patients had a prothrombin time well prolonged above that of the average (22.9 seconds).

Eleven of the 71 patients studied had prothrombin coagulation times over thirty seconds. Included in this group were two essentially normal females, one patient five months pregnant, one patient with cardiac failure, one patient with cirrhosis of the liver, one patient with chronic ulcerative colitis, one patient with pancreatitis, and four post-partum patients that were still hospitalized.

Russell viper venom is available as a standardized preparation. It simplifies the performance of prothrombin coagulation tests and readings of the end point of fibrin web formation are easier than when tissue extracts of variable potency are used.

CONCLUSIONS

1. Prothrombin coagulation tests were performed on 71 patients with Quick's method and a modified Quick's method using Russell viper venom.

2. The time differences between the two tests were found to be very small and were not statistically significant.

3. The use of Russell viper venom made the performance of the test and the accurate reading of the end point of formation of fibrin web simpler than when tissue extract was used.

REFERENCES

1. Cheney, G.: The Plasma Coagulation Time as a Simple Test for Vitamin K Deficiency, *Am. J. M. Sc.* 200: 327, 1940.
2. Fisher, R. A., and Yates, F.: Statistical Tables, Edinburgh and London, 1938, Oliver and Boyd.
3. Fullerton, H. W.: Estimation of Prothrombin, A Simplified Method, *Lancet* 2: 195, 1940.
4. Kato, K., and Poncher, H. G.: The Prothrombin in the Blood of Newborn Mature and Immature Infants, As Determined by the Micro Prothrombin Test, *J. A. M. A.* 114: 749, 1940.
5. Quick, A. J.: The Nature of the Bleeding in Jaundice, *J. A. M. A.* 110: 1658, 1938.
6. Smith, H. P., Ziffren, S. E., Owen, C. A., and Hoffman, G. R.: Clinical and Experimental Studies on Vitamin K, *J. A. M. A.* 113: 380, 1939.

A NEW METHOD FOR DETERMINING SPECIFIC GRAVITY OF BLOOD AND BODY FLUIDS*

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FOR the determination of the specific gravity of blood the exact method of pyknometry can be employed, but on account of its convenience, Hammerschlag's method¹ is often used instead in clinical practice. The specific gravity of colorless body fluids is usually determined by refractometry, based on protein content. Herein is discussed a very accurate method which permits determination of the specific gravity of blood or other body fluids by making use of a small quantity (one to two drops) of blood, exudate, transudate, etc.

In Hammerschlag's method a mixture of chloroform and benzene is employed, the specific gravity of which approximates the expected specific gravity of the blood to be examined. Into this mixture one drop of blood is dropped; if it sinks to the bottom of the vessel, chloroform is added; if it floats on the surface, benzene is added, stirring the whole time, till the drop keeps floating in the middle of the mixture. This indicates that the specific gravity of the blood and mixture is equal. At this concentration the specific gravity of the mixture and hence of the blood is determined by an areometer.

The new method is a simplification of the Hammerschlag method and is carried out by adding one drop of the liquid to be examined (for simplicity's sake blood will be taken as an example), to a mixture of chloroform and benzene contained within a previously calibrated vessel. If the drop of blood sinks to the bottom, chloroform is added; if it floats on the surface, benzene is added, continuously stirring, till the drop floats at the center of the mixture. The specific gravity can then be directly read off from the graduations on the neck of the vessel.

*From the Jewish Hospital, Budapest.

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The vessel (Fig. 1) of the blood gravimeter consists of a globe-shaped body and a long slender neck, closed by a glass stopper. Its volume up to the first mark is equal to 25 c.c., or a multiple of this quantity, and is marked "50." Assuming that the gravimeter is filled up to this mark by a chloroform-benzene mixture of a specific gravity 1.050 and a volume of 25 c.c., it follows by the equation $25 \times 1.050 + 1.476x = 1.055 \times (25 + x)$ that 0.297 c.c. of chloroform has to be added to raise the specific gravity to 1.055. Similar calculation discloses the quantity of chloroform necessary to increase the specific gravity to 1.060, 1.065 . . . 1.090. The values calculated are as follows:

SPECIFIC GRAVITY	CORRESPONDING VOLUME
1.050	25.000
1.055	25.297
1.060	25.601
1.065	25.912
1.070	26.231
1.075	26.558
1.080	26.893
1.085	27.237
1.090	27.590

By the equation $25 \times 1.050 + 0.884y = 1.045 \times (25 + y)$ it is possible to calculate the amount of benzene to be added to the 25 c.c. of originally 1.050 mixture, that the latter may decrease to the specific gravity of 1.045, 1.040 . . . 1.030. The values calculated are as follows:

SPECIFIC GRAVITY	CORRESPONDING VOLUME
1.050	25.000
1.045	25.776
1.040	26.602
1.035	27.484
1.030	28.425

The calibration of the blood gravimeter is estimated by using the data of the two columns above. On the one side of the neck of the vessel those volumes are marked which correspond to that amount of chloroform which, when added to the mixture with the original specific gravity of 1.050, increases this up to 1.055, 1.060 . . . 1.090, respectively. On the other side, those volumes are marked, corresponding to which such amounts of benzene have been added that the original specific gravity of the mixture is decreased to 1.045, 1.040 . . . 1.030. It is of advantage to mark instead of the volumes themselves, the corresponding specific gravity values. If the distance between two of the marks is subdivided into five equal parts, differences of one degree of specific gravity can be determined.

The calibrated gravimeter is used as follows: The vessel is filled up to the 50 mark with a chloroform-benzene mixture of 1.050 specific gravity.* The tip of a finger is cleansed and pierced, and one or two drops of blood are sucked up into a thin pipette. After the tip of the pipette has been cleaned, one drop of blood is dropped into the mixture. If the specific gravity exceeds 1.050, the drop sinks to the bottom. While cautiously rotating the closed vessel, chloro-

*The specific gravity of chloroform and benzene being known (1.476 and 0.884, respectively at 20° C.) and marking it 1476 or 884, respectively, the proportion necessary to produce a mixture of 1.050 calculated by the equation $1476x + 884 \times (1 - x) = 1.050 \times 100$ is 28:72. Consequently, 28 c.c. of chloroform and 72 c.c. of benzene are necessary to produce 100 c.c. of a chloroform-benzene mixture of 1.050 specific gravity.

form is added in small quantities till the drop of blood floats at the center of the mixture contained in the globular part of the vessel. At this concentration the specific gravity is read at the graduation. On the other hand, if the specific gravity is less than 1.050, benzene is added to the mixture until the drop floats in the middle.

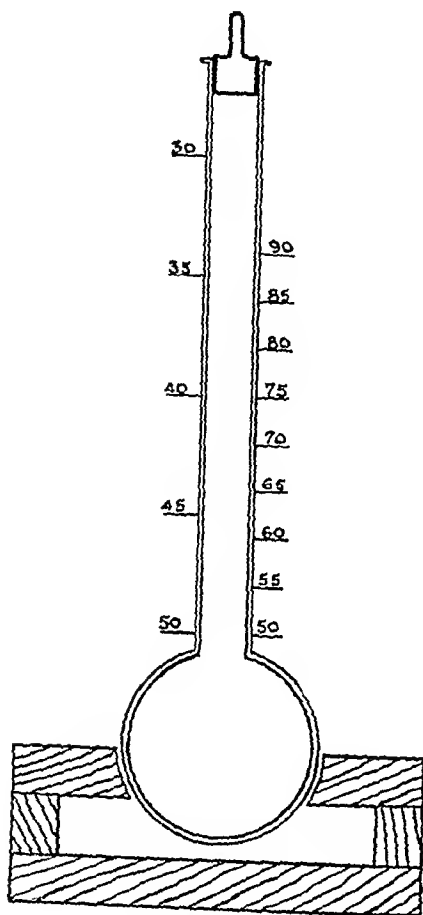


Fig. 1.

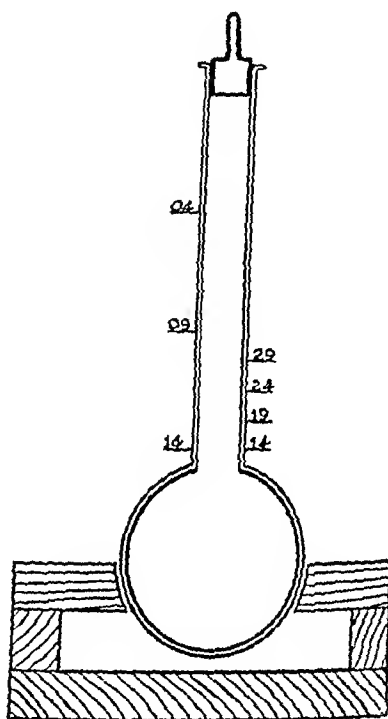


Fig. 2.

Allowing that the specific gravity of chloroform and benzene used for the determination is taken at 20° C., and that the coefficient of the thermal expansion of both^{2, 3} is equal to 0.001, the temperature of the mixture has to be determined after use and has to be corrected accordingly. This is done by subtracting as many points of the specific gravity determined as are above 20, supposing that the temperature has been found to exceed 20° C. Contrariwise, as many points are added as the temperature has been found to be below 20° C.

The method is also suitable for determining the specific gravity of other body fluids. Considering that the specific gravity of body fluids is for the most part below 1.050, another gravimeter (see Fig. 2) has to be calibrated which permits the reading of specific gravity values between 1.029 and 1.004. As a starting point a chloroform-benzene mixture of 1.014 specific gravity is suitable, since it is easy to procure 25 c.c. of it, by mixing 5.5 c.c. of chloroform and 19.5 c.c. of benzene as indicated by the equation $1.476x + 0.884 \times (1 - x) = 1.014$.

By the equation $25 \times 1.014 + 1.476x = 1.019 \times (25 + x)$ the amount of chloroform necessary to add to the mixture to arrive at a specific gravity of 1.019, 1.024 . . . 1.029 is calculated. The values are as follows:

SPECIFIC GRAVITY	CORRESPONDING VOLUME
1.014	25.000
1.019	25.263
1.024	25.543
1.029	25.829

By the equation $25 \times 1.014 + 0.884y = 1.009 \times (25 + y)$ the amount of benzene can be calculated by the addition of which the specific gravity of the subsequent mixtures can be regulated at 1.009 and 1.004. The values calculated are as follows:

SPECIFIC GRAVITY	CORRESPONDING VOLUME
1.014	25.000
1.009	26.000
1.004	27.083

Allowing that the specific gravity of the body fluids, which may have to be determined, varies between the values of 1.004 and 1.090, two separate calibrated vessels of which the one is graduated from 1.004 to 1.029 and the other from 1.030 to 1.090 suffice for determining the specific gravity of body fluids encountered in clinical practice.

The figures obtained by the blood gravimeter were controlled several times by comparing them with the values obtained by pyknometry. The difference found never exceeded one to two points.

SUMMARY

A new method suitable for simple and reliable determination of the specific gravity of blood and body fluids has been described. Although differing only slightly from the results obtained by pyknometry, this method is simple and easy to use, and is especially valuable for making estimations in a large number of cases. The simplicity of the method permits its use by the clinician at the bedside. One to two drops of the substance to be examined are sufficient. Besides the previously calibrated gravimeter, only chloroform, benzene, and a thermometer are necessary to practice it. The low cost of the method is a further advantage. Besides its clinical application the method is sufficiently reliable to be used for comparative investigations of the change of specific gravity in different types of disease and may, therefore, be used with advantage for scientific purposes also.

REFERENCES

1. Hammerschlag, A.: *Ztschr. f. Klin. Med.* 20: 444, 1892.
2. Herz, W., and Rathmann, W.: *Chemische Zeitung* 41: 146, 1917.

A NOTE ON THE VISSCHER-BOWMAN CHEMICAL TEST FOR PREGNANCY

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THE Visseher-Bowman test¹ was thought by some to be of value in the diagnosis of early pregnancy.¹ It is performed by adding one drop of 1 per cent hydrogen peroxide, five drops of 1 per cent phenylhydrazine hydrochloride, five drops of 5 per cent methyleyanide, and five drops of concentrated hydrochloric acid to 1 c.c. of urine, then heating in a water bath for twenty-five minutes. A flocculent brownish-red precipitate is indicative of a positive reaction.

Sheehan² decided that the effect of hydrochloric acid with heat was responsible for the positive reaction and found that over 60 per cent of male urine gave positive results. These results agree with Krieger,³ who considered the reaction due to nonspecific reducing substances and not to the hormones of the anterior lobe of the pituitary. These nonspecific substances appeared to be reducing carbohydrates, particularly in the presence of albumin, urinary pigments, and other reducing substances, especially if concentrated.

The urines of 232 persons were tested, 35 being urines of pregnancy and the remaining being urines of 77 males and 120 nonpregnant females. The results showed that there were as many positive reactions in the males as in females. About 75 per cent of the total group were positive. Apparently the test does not show the presence of hormone nor is it a specific test for pregnancy. The length of time of boiling was not found to influence greatly the results, except that where the boiling was continued longer, a greater percentage of positive reactions was obtained in both males and females. Apparently the reaction was produced by nonspecific substances in the urine, as a greater percentage of positive results were obtained from normal urine and from urines of persons with diabetes by adding solutions of dextrose.

CONCLUSION

This test appears to be of no value because nearly as many positive results were obtained from the urines of males and nonpregnant females as from the urines of pregnant females.

REFERENCES

1. Visscher, J. P., and Bowman, D. E.: Chemical Determination of Pregnancy, *Proc. Soc. Exper. Biol. & Med.* 31: 460, 1934.
2. Sheehan, J. F.: The Visscher-Bowman Chemical Test for Pregnancy and the Urinary Pigment, *Surg., Gynec. & Obst.* 64: 806, 1937.
3. Krieger, V. I.: An Investigation of the Visscher-Bowman Test for Pregnancy, *M. J. Australia* 1: 463, 1939.

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A SIMPLE AND INEXPENSIVE MICROTITRATION APPARATUS*

ESPECIALLY USEFUL FOR TITRATING CULTURE MEDIA

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THE need for a simple, yet accurate and convenient, apparatus for titrating culture media resulted in our design. It is especially useful for making the titration necessary for the adjustment of the reaction of culture media by the colorimetric method, although it may be used for any titration where small amounts of exact alkali or acid are required. It is equally useful also in the adjustment of the reaction of culture media by the Fuller method.

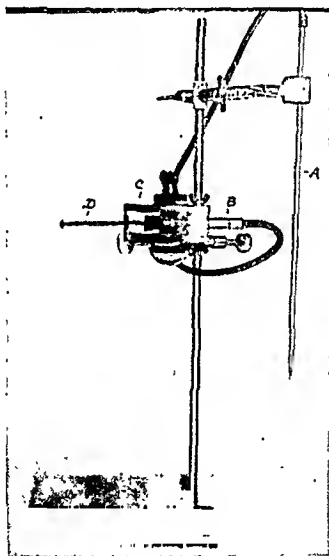


FIG. 1.

The apparatus consists of an ordinary ring stand, two clamps, a 1 c.c. pipette graduated in hundredths, a 2 c.c. hypodermic syringe, and a short piece of rubber tubing. The pipette (A) is held in a vertical position by an ordinary burette clamp. The pipette is connected by rubber tubing to the syringe (B) held in a horizontal position in a simply constructed wooden block (C) by a second burette clamp. The wooden holder for the syringe is a split block of wood

*From the Departments of Bacteriology and Physiology, School of Medical Sciences, Wake Forest College, Wake Forest.

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through which a hole, just large enough to accommodate the barrel, has been bored. The two portions of the split block are held together by bolts with wing nuts. A square of tin to which a nut has been soldered is tacked across one end of the block; a long screw (*D*) is threaded through this nut and is fastened to the plunger of the syringe by one end of a small one-hole rubber stopper, to which it is glued, and into which the screw is threaded at the opposite end. The same arrangement may be made merely by sinking the nut through which screw (*D*) passes into the block. The level of the fluid within the pipette is regulated by turning the screw (*D*) which forces the plunger in or out. A feature of some value is that the syringe is on the same level as the upper figures on the pipette; so that both are on the same level with the eyes when the instrument is being used.

Our use of the instrument has indicated that it has the distinct advantage of being more accurate than manipulation of the pipette by hand in the usual way, since the expulsion of fluid from the pipette is always under positive and complete control. If necessary, a fraction of a drop may be used with ease, a thing which is rather difficult without this control.

The apparatus may be constructed at a very moderate cost. A ring stand may be purchased for 45 cents, clamps for 35 cents each, syringe for \$1.25, and the pipette for 25 cents. The wooden block can be made in the shop in a few minutes at almost no cost. The total cost would be approximately \$2.65.

A SIMPLE ABSORPTION BULB FOR USE WITH AN ORDINARY SYRINGE IN DETERMINING OXYGEN OF THE AIR IN AN OXYGEN TENT*

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USUALLY it is not necessary to determine the oxygen content of the atmosphere of the oxygen tent with the precision attainable with the more refined gas analysis apparatus. Numerous simple forms of apparatus have come into use. Henderson and Greenberg¹ employed an ordinary syringe with simple accessories. The reagent for the absorption of oxygen was a solution containing sodium hydrosulfite, which was drawn into the syringe after measurement of the volume of the sample. Hoechstetter² used an apparatus somewhat similar in principle, consisting of two syringes, the lower one of which contained copper screen and a solution of ammonia and ammonium chloride. In each of these devices, the volume of the sample was measured at a pressure dependent upon the weight of either the barrel of the syringe and its accessories or the plunger. After absorption of oxygen the volume of the residual gas was measured supposedly under the same pressure. However, in practice, using Henderson's apparatus, we found it difficult to eliminate entirely sticking of

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the plunger, and since there was no way of measuring the pressure of the gas in the syringe, we were never certain that we had obtained the same pressure for both readings. While it might be possible to select a syringe free of any tendency to stick, it is obviously advantageous to be able to use any syringe that happens to be at hand. By means of the manometer fused to the absorption bulb shown in Fig. 1., the operator is enabled to bring the residual gas to the same pressure as the original sample, despite any resistance that is likely to occur.

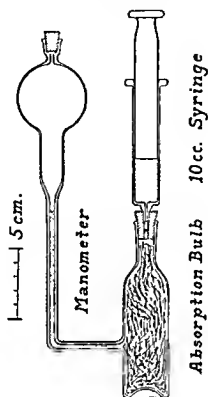


Fig. 1.

The apparatus consists of an ordinary syringe, preferably of 10 c.c. capacity, an absorption bulb of about 60 c.c. capacity, and a manometer with an expansion bulb as shown. The sizes of parts may be obtained by reference to the accompanying scale, but these dimensions are not critical. The small tubing constituting the manometer proper extends to a point above the top of the stopper in the absorption bulb. This one-hole rubber stopper and the solid stopper at the top of the manometer are indicated by diagonal shading. The neck of the absorption bulb is tapered to fit a O or OO stopper, thus insuring a snug fit and a minimum change in volume due to distortion of the stopper in inserting the tip of the syringe. The apparatus is supported in a hole in a wooden block.

To prepare the bulb for use, light copper turnings are stuffed into it through the neck, and the solution is poured onto the turnings until it reaches the level of the bottom of the stopper. The stopper is then inserted, and a small piece of glass rod is used to plug the hole. Any excess solution may be rinsed off from the outside of stopper or bulb. The copper turnings should fill the bulb, but should not be packed tight enough to impede displacement of all gas bubbles by the solution after absorption. The solution is made according to Badger³ by saturating a mixture of one part of concentrated ammonium hydroxide and two parts of water with ammonium chloride. When the apparatus is not in use, the top of the manometer is closed by a solid rubber stopper to prevent slow absorption of oxygen from the air. This stopper is removed during analyses.

A determination is made in the following manner: A representative sample of more than 10 c.c. is obtained in the syringe. The plunger is brought to the 10 c.c. mark and held rigidly in this position while the tip of the syringe is inserted into the hole in the stopper and while the level of liquid in the manometer is marked by a glass-marking pencil or by a small clamp placed on the manometer. The plunger is then pushed down slowly until the sample has been forced into the bulb; it is held there for a few seconds, and then it is lifted until the liquid in the bulb touches the bottom of the stopper. This operation is repeated until the volume of the residual gas is constant. Usually four transfers of the sample into and out of the bulb are enough. The plunger is finally raised (or lowered) until the liquid in the manometer has been brought back to the original mark, and the volume of the residual gas is read on the graduations on the syringe. The difference between the initial reading (10 c.c.) and this final reading represents the volume of oxygen absorbed.

Theoretically, there should be some error due to the absorption of oxygen from the lumen of the tip of the syringe and from any small bubble that may be present in the hole of the stopper, but actually this error is too small to detect. An expected error due to change in level of the liquid when the tip of the syringe is pushed into the stopper is also too small to be detected. Usually no error is introduced by a rather large bubble below the stopper because it consists of inert residual gas from which the oxygen has been absorbed. Determination of the oxygen in air or in known mixtures of air and pure oxygen are usually accurate to within 1 or 2 per cent of the total volume of the sample.

Although the open manometer is less compact and offers less than complete protection of the solution from contact with the outside air, it is superior to a closed manometer in ease of manipulation and in freedom from the effects of changes in temperature.

A satisfactory clamp for marking the position of the meniscus in the manometer at the time of the first reading may be made by opening out a paper clip until it can be placed over the glass tubing, and then bending the long loop around the tube to prevent its slipping off.

REFERENCES

1. Henderson, Yandell, and Greenberg, L. A.: Gas Analysis With an All-Glass Syringe for Pneumonia Tents, *J. A. M. A.* 96: 1474, 1931.
2. Hoechstetter, S. S.: A Simple Oxygen Analyser for Use in Oxygen Tent Therapy, *Mil. Surgeon* 85: 384, 1939.
3. Badger, W. L.: Determination of Oxygen by the Copper-Ammonia-Ammonium Chloride Reagent, *Indust. & Engin. Chem.* 12: 161, 1920.

A SIMPLIFIED DETERMINATION OF BLOOD PROTHROMBIN LEVELS IN THE NEWBORN*

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RECENT studies have aroused interest in the relationship of vitamin K to various hemorrhagic tendencies in the human being. In the adult these have been largely concerned with bleeding states in obstructive jaundice.¹⁻³ In the newborn infant they have been concerned particularly with hemorrhagic disease. The importance of vitamin K in the establishment and maintenance of adequate prothrombin levels in the blood has been verified.

Study of the prothrombin content of the blood of newborn infants has shown that considerable variability exists in the normal infant during the first week of extrauterine existence.⁴⁻⁸ In general, this is manifested as a drop in prothrombin level during the first few days of life followed by a return to at least the original level during the second week.

Routine study of a large series of infants has been complicated by the necessity of repeated venepuncture to obtain blood for study. More recently, Bray and Kelley⁹ and Kato¹⁰ have reported the use of capillary blood obtained by heel puncture as giving satisfactory results. During the past nine months we have been using a modification of the Smith¹¹ bedside test as a simple clinical method for evaluating the prothrombin factor in blood of the newborn.

METHOD

A total of 20 c.mm. of thromboplastin extract are placed on a clean glass slide. Blood is obtained from the infant to be tested by heel puncture. Twenty cubic millimeters of blood are drawn into a blood pipette and immediately transferred to the drop of thromboplastin on the slide. A stop watch is started at this time. The blood and thromboplastin solution are mixed, and the drop is pricked every second with a stylus until a firm, fibrin strand adheres to the point. The interval in seconds is recorded as the prothrombin clotting time. A normal control is run in the same manner using adult finger blood. The prothrombin level is expressed in per cent of normal using the formula suggested by Smith and co-workers:

$$\frac{\text{Clotting time of control}}{\text{Clotting time of unknown}} \times 100 = \text{clotting activity in per cent of normal.}$$

The technique of making the heel puncture which was found to give the best results is as follows: The infant is cradled on the left arm while holding the infant's left foot in the left hand. The heel is sponged with alcohol and dried carefully. Using a No. 11 knife blade the puncture is made slowly and deliberately on the posteromedial edge of the heel at a depth of approximately

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$\frac{1}{8}$ inch. The left leg is then lowered into a dependent position, and a drop of blood immediately begins to form. The infant is replaced in the crib and blood is drawn into a pipette to the 20 c.mm. mark. Delay in obtaining the blood and squeezing the heel will shorten the prothrombin clotting time. A second determination cannot be made from the same bleeding point as the blood will always clot considerably quicker than the first drop obtained.

The thromboplastin extract is prepared in the following manner: Fresh sheep or swine lung is ground in a domestic meat grinder and placed in a large beaker. To this is added 1 c.c. of normal saline per gram of ground lung. The lung-saline mixture is stored overnight in the refrigerator. It is then strained through a double layer of broadcloth. The saline extract is placed in small test tubes and securely corked. These tubes are stored in the freezing compartment of a refrigerator where the thromboplastin extract retains its potency for at least six weeks. When ready for use, a tube is allowed to thaw and the extract is used at room temperature.

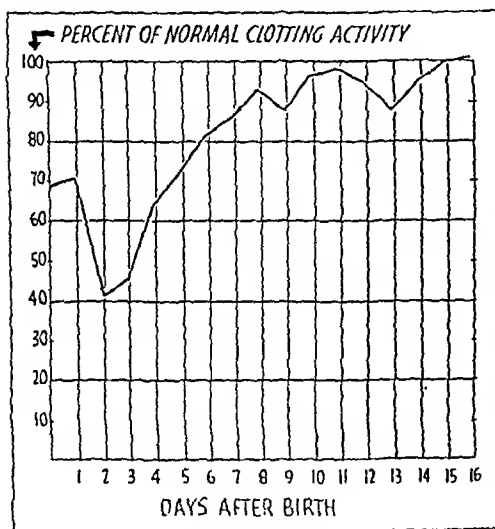


Chart 1.

Chart 1.—Average values in per cent of normal clotting activity on the various days of the neonatal period in 114 infants.

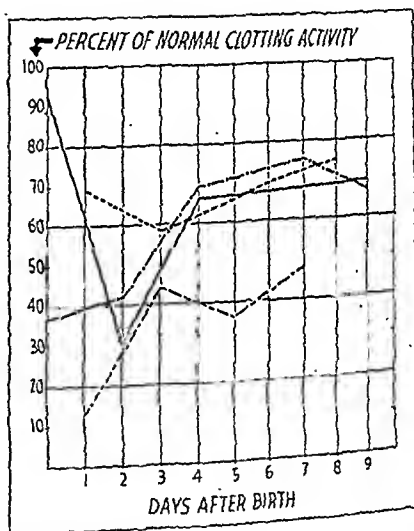


Chart 2.

Chart 2.—Curves showing the variation in clotting activity in four normal infants.

RESULTS AND COMMENT

We recognize that with this method we are not obtaining an accurate determination of the prothrombin level of the blood, but rather a summation of several factors, including particularly the amount and convertibility of prothrombin. We feel that for practical purposes such a measure of the ability of the blood to clot in the presence of an excess of thromboplastin will indicate those infants in whom a critical deficiency exists.

Using this method we have routinely determined the clotting activity in a series of 114 infants. The infants were tested every other day during the first ten days of life, or for longer periods if they remained in the hospital. Chart 1 illustrates in per cent of normal clotting activity the average level for each day of life in the total group of 114 infants. It will be noted that the lowest levels

are reached forty-eight to seventy-two hours after birth. By the end of the first week the average values are well within the normal range and seem to be maintained at that level.

Considerable variation exists in individual infants as shown in Chart 2. The four infants whose curves are illustrated were all full term, delivered without incident, breathed spontaneously, and were breast fed. In reviewing our charts we find no evidence of consistent differences in the clotting activity of premature and full-term infants. We do, however, find suggestive evidence that there is a seasonal variation in the type of curve. In 10 infants delivered consecutively during November, 1939, the average low reading was 45 per cent, while in 10 infants delivered during March, 1940, the average low reading was 12 per cent of normal clotting activity. This observation is not surprising in view of the probable differences in diet of the average patient during the summer and winter months. Further study of this phase is being made.

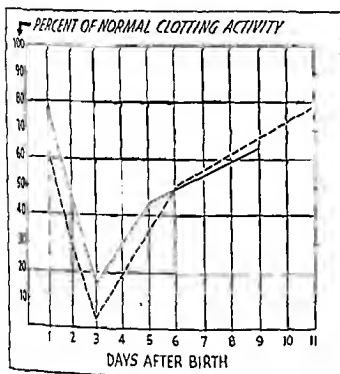


Chart 3.

Chart 3.—Typical curves illustrating the marked drop in clotting activity in the blood of some normal infants.

Chart 4.—A prolonged low prothrombin level in an infant with a bleeding tendency showing a rapid response to a vitamin K preparation.

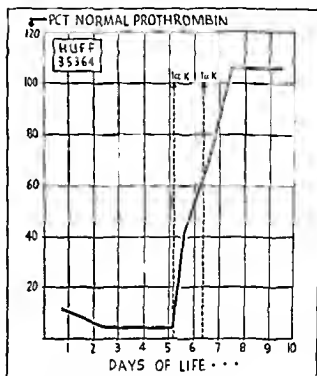


Chart 4.

Two typical curves of a different type are shown in Chart 3. The striking drop on the third day is obvious. Both of these infants were normal, and there was a rather prompt return of the prothrombin clotting time to normal. It is suggested that any unusual trauma to infants showing such a curve might well precipitate serious bleeding. This may be a factor in increasing cerebral injury associated with difficult delivery or prolonged asphyxia, particularly where an increase in the symptoms appears twenty-four to seventy-two hours after delivery.

That vitamin K will correct this decrease in clotting activity and that, if administered early, it will prevent the drop is indicated by recent reports in the literature,^{6,7} as well as by investigations in progress on our service. In Chart 4 we illustrate the rapid rise to normal level of the clotting activity in an infant who, coincident with the low levels found during the first five days of life,

passed bloody mucus from the bowel and vomited a blood-streaked material. Therapy consisted of 1 mg. of 2-methyl-1, 4-naphthoquinone (Squibb) in corn oil, given orally on the fifth and sixth days as shown. No further bleeding appeared after administration of this material.

The apparent relationship of a low prothrombin content of the infant's blood to hemorrhagic disease of the newborn, and very probably to other bleeding tendencies, indicates the desirability of an increasing number of observations of this relationship. This is particularly true in view of the reported efficacy of vitamin K in the therapy of these infants. It is with this thought in mind that we are presenting a simplified procedure for clinical evaluation of the prothrombin level. It can be readily performed without special laboratory facilities and does not require venipuncture on the infant.

CONCLUSIONS

1. A simplified procedure for the determination of prothrombin levels in the blood of the newborn infant is described.
2. With this procedure a decrease in clotting activity is demonstrated and reaches a maximum the second and third days of the neonatal period.
3. In the normal infant there is a spontaneous return to normal clotting activity by the beginning of the second week of life.
4. A seasonal variation in the degree of prothrombin deficiency is suggested. This appears to be most marked during the early spring.

REFERENCES

1. Warner, E. D., Brinkhaus, K. M., and Smith, H. P.: Bleeding Tendency of Obstructive Jaundice: Prothrombin Deficiency and Dietary Factors, *Proc. Soc. Exper. Biol. & Med.* 37: 628, 1938.
2. Quick, A. J.: The Nature of Bleeding in Jaundice, *J. A. M. A.* 110: 1658, 1938.
3. Butt, H. R., Snell, A. M., and Osterberg, A. E.: The Use of Vitamin K and Bile in Treatment of the Hemorrhagic Diathesis in Cases of Jaundice, *Proc. Staff Meet., Mayo Clin.* 13: 74, 1938.
4. Quick, A. J., and Grossman, A. M.: Concentration of Prothrombin in the Blood of Babies (Three to Seven Days Old), *Proc. Soc. Exper. Biol. & Med.* 40: 647, 1939.
5. Brinkhaus, K. M., Smith, H. P., and Warner, E. D.: Plasma Prothrombin Level in Normal Infancy and in Hemorrhagic Disease of the Newborn, *Am. J. M. Sc.* 193: 475, 1937.
6. Waddell, W. W., Jr., and Guerry, DuPont, III: Effect of Vitamin K on the Clotting Time of Prothrombin and the Blood, With Special Reference to Unnatural Bleeding of the Newly Born, *J. A. M. A.* 112: 2259, 1939.
7. Hellman, L. M., and Shettles, L. B.: Factors Influencing Plasma Prothrombin in the Newborn Infant, *Bull. Johns Hopkins Hosp.* 65: 138, 1939.
8. Kato, K., and Poncher, H. G.: The Prothrombin in the Blood of Newborn Mature and Immature Infants, *J. A. M. A.* 114: 749, 1940.
9. Bray, W. E., and Kelley, O. R.: Prothrombin Studies, Especially in the Newborn, *Am. J. Clin. Path.* 10: 154, 1940.
10. Kato, K.: Micro-Prothrombin Test With Capillary Whole Blood, *Am. J. Clin. Path.* 10: 147, 1940.
11. Ziffren, S. E., Owen, C. A., Hoffman, G. R., and Smith, H. P.: Control of Vitamin K Therapy. Compensatory Mechanisms at Low Prothrombin Levels, *Proc. Soc. Exper. Biol. & Med.* 40: 595, 1939.

ON THE MEASUREMENT OF EXTRACELLULAR FLUID VOLUME WITH THIOCYANATE AND BODY FLUID ANALYSES IN 33 NORMAL INDIVIDUALS*

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IN 1934 Crandall and Anderson¹ reported results of studies on determination of body fluid available for solution of thiocyanate. They concluded that consistent results could be obtained in the same individual, and that with qualifications the solvent measured could be considered extracellular fluid. The thiocyanate ion when injected intravenously as sodium thiocyanate, or when taken by mouth as the sodium or potassium salt, diffused rapidly into water of plasma, red blood cells, transudates, and gastrointestinal secretions but not into cerebrospinal fluid. The thiocyanate was found to be excreted from the body quantitatively through the urine. Laviètes, Bourdillon, and Klinghoffer² showed that thiocyanate, sucrose, and inorganic sulfate are distributed through approximately the same fraction of body fluid. This fraction was found to make up about 20 per cent of the weight of normal persons, and was considered as composed in all probability chiefly of extracellular fluid. These workers discovered that thiocyanate becomes distributed in serum partly in bound form, and devised a formula to correct for consequent unequal distribution in calculating extracellular fluid volume. Gregersen and Stewart³ developed a technique for simultaneous determination of plasma volume and extracellular fluid volume, based on the spectrophotometric determination of concentrations of the blue dye of Gregersen's plasma volume method⁴ and the thiocyanate of the extracellular fluid volume method.

We have used the thiocyanate method extensively for determining extracellular fluid volume in normal subjects and in patients and animals with disturbances in water balance. The present study deals with technique we now use in making the determination, and reports illustrative normal values.

METHODS

Reagent grade sodium thiocyanate is dried at 100° C. until constant in weight. Fifty grams are weighed out, dissolved in 500 c.c. of distilled water, and the solution is filtered. The filter paper and funnel are washed with 250 c.c. of warm distilled water. The solution, then having a volume of about 750 c.c., is sterilized by boiling in a liter, covered, volumetric flask for thirty minutes. After cooling, the solution is made up to volume with sterile distilled water. The solution is put up in 25 c.c. quantities in sterile test tubes capped

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with Davol baby bottle caps No. 150. Before using the solution, 25 c.c. are given to a dog intravenously, and a precautionary bacteriologic culture of a sample is made to exclude chance contamination. The solution keeps several months without deterioration. On using it the solution must be perfectly clear.

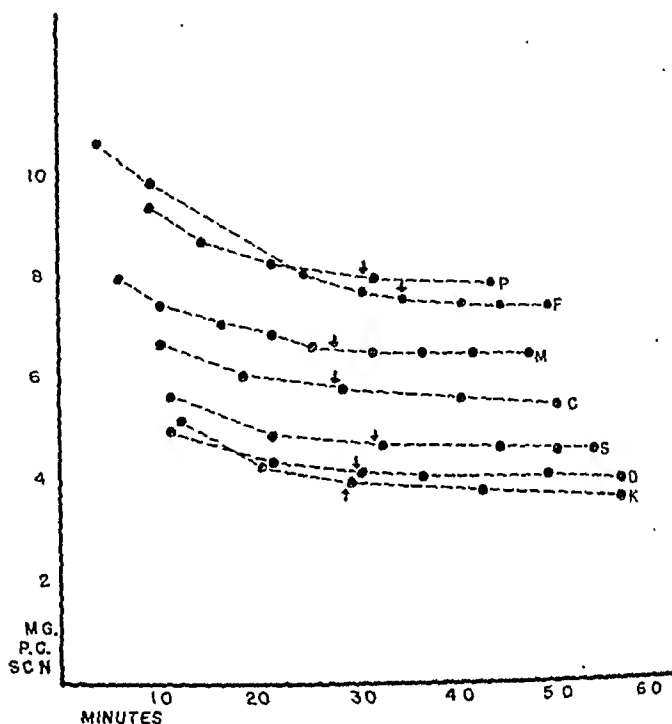


Chart 1.—Thiocyanate diffusion curves after intravenous injection in normal individuals, constructed from plasma concentrations.

For injecting the thiocyanate solution into a vein a calibrated 10 c.c. or 20 c.c. syringe is used, the syringe and needle having been sterilized by autoclaving. The syringe and needle are washed with three or four 2 c.c. quantities of blood before withdrawing the needle from the vein. A vein in the opposite arm is used in taking samples after this injection, and five samples of blood of 5 c.c. each are taken, preferably at intervals of ten; twenty, thirty, forty, and fifty minutes. In making the determination in adults as a rule 20 c.c. of the 5 per cent solution are given. In children or in dogs, and in adults upon whom the determination has previously been made within three days, 10 c.c. are injected.

In samples of serum withdrawn subsequently thiocyanate concentration is measured in a colorimeter, using the method of Crandall and Anderson,¹ or the spectrophotometric method of Gregersen and Stewart.³ In either case the filtrate obtained after precipitation of serum protein must be clear before producing color by addition of the ferric nitrate reagent. If thiocyanate has been given within a week, a pre-sample must be taken and its thiocyanate concentration deducted in using the colorimetric technique. In the spectrophotometric measurement the thiocyanate content of the pre-sample is automatically deducted, since the determination measures increase in optical density

of the post-sample compared with the pre-sample. We now use only the intravenous technique in giving sodium thiocyanate, since we have found the results much more consistent than with the oral method. In no case has evidence of toxic reaction to the injected sodium thiocyanate been observed. However, we never inject more than 20 c.c. of 5 per cent sodium thiocyanate at one time, and have not produced levels higher than 15 mg. per cent in the blood.

CALCULATIONS

An important point in technique relates to the diffusion curve which is constructed after the intravenous injection of sodium thiocyanate. For the first fifteen to twenty-five minutes the concentration in the blood serum falls rapidly. The curve then assumes a different form, and the subsequent gradual decline in serum concentration depends on urinary excretion. To get accurate results consistently we find it necessary to obtain enough samples of serum to plot the two components of the curve. The point at which the two components merge, indicated by the arrow in Chart 1, is then considered as the concentration in the serum after uniform diffusion in extracellular fluid has resulted. Results may be quite variable if only a single reading is made thirty or forty minutes after injection, for in abnormal conditions, such as deranged water balance and anesthesia, the second component of the curve, as well as the first, may have a variable slope. This point is indicated in the series of curves shown in Chart 1.

In calculating fluid available for solution of thiocyanate, considered as extracellular fluid plus water of red blood cells, we use the simple formula (formula A):

$$\text{c.c.} = \frac{\text{mg. SCN given} \times 100}{\text{mg. \% concentration SCN in serum}}$$

This uncorrected formula gives results of practical value, though it ignores such considerations as partial binding of thiocyanate by serum protein and lower concentration in red blood cells than in serum due to lower water content in cells. If plasma volume and hematocrit have been determined at the same time as extracellular fluid volume, then the formula of Lavietes, Bourdillon, and Klinghoffer² may be used (formula B):

$$\frac{\text{Extracellular fluid volume} = (\text{mg. SCN retained in body}) - (\text{conc. in serum} \times \text{blood vol.}) + (\text{serum vol.})}{(100/110) (\text{conc. in serum})}$$

This formula introduces too great an error if serum volume and whole blood volume are assumed to be constant fractions of body weight, particularly in the conditions of deranged hydration where the determination would be of most interest. There is undoubtedly error also in computing whole blood volume from plasma volume and hematocrit of venous blood, due to the higher plasma content of capillary blood resulting from axial streaming of corpuscles.

In Table I are presented data from 33 normal physicians and students, and patients awaiting elective surgical procedures, studied in the resting post-absorptive state. Average values for the group are computed. Values for plasma volume⁴ and extracellular fluid volume are expressed in cubic centi-

TABLE I
BODY FLUID DATA OBTAINED IN 33 NORMAL PEOPLE

body fluid data obtained in 33 normal people

AGE-SEX	WEIGHT	SURFACE AREA	HEMATOCRIT	PLASMA VOLUME				BLOOD VOLUME				EXTRACELLULAR FLUID VOLUME (FORMULA A)				EXTRACELLULAR FLUID VOLUME (FORMULA B)				PLASMA PROTEIN		SERUM SODIUM		SERUM CHLORIDE		OXYGEN CAPACITY	
				c.c.	Kg.	L./Sq.M.	c.c./Kg.	L./Sq.M.	c.c.	Kg.	L./Sq.M.	c.c./Kg.	L./Sq.M.	c.c.	Kg.	L./Sq.M.	c.c.	Kg.	L./Sq.M.	Gm.	%	Gm.	%	Meq./L.	L.	Meq./L.	L.
1 42-M	56.7	1.61	44.7	2,964	52.2	1.84	5,360	94.5	3.3	10,140	179	6.31	8,219	7.17	212	141.6	105.6	19.1	7.17	212	141.6	105.6	19.1	1,024			
2 22-M	66.4	1.80	47.2	3,633	54.7	2.02	6,880	103.7	3.8	12,520	188	6.96	9,820	7.03	256	135.2	104.2	19.1	7.03	256	135.2	104.2	19.1	1,314			
3 56-M	59.37	1.71	43.3	3,265	54.4	1.91	5,760	97.1	3.4	12,370	203	7.23	10,540	7.98	260	140.0	102.1	18.7	7.98	260	140.0	102.1	18.7	1,077			
4 28-M	60.0	1.71	43.0	2,421	40.4	1.42	4,249	70.7	2.5	11,470	246	8.63	13,940	7.20	174	139.4	107.0		7.20	174	139.4	107.0					
5 34-M	56.0	1.55	47.0	2,408	43.1	1.55	4,546	81.3	2.9	9,940	178	6.42	8,340	7.64	184	137.9	101.5		7.64	184	137.9	101.5					
6 48-M	70.0	1.78	45.4	2,928	41.8	1.61	6,555	93.8	3.7	12,570	179	7.07	9,510	7.46	219	138.0	101.3	16.4	7.46	219	138.0	101.3	16.4	1,075			
7 16-M	44.8	1.42	41.5	1,758	39.2	1.24	3,905	67.1	2.1	13,140	183	8.25	15,900	7.17	263	137.3	98.0	20.7	7.17	263	137.3	98.0	20.7	1,453			
8 17-M	99.2	2.20	47.7	3,670	36.9	1.67	7,020	70.8	3.2	13,970	191	6.98	11,070	8.17	311	139.7	100.3	19.9	8.17	311	139.7	100.3	19.9	1,472			
9 27-M	73.2	2.00	48.5	3,810	52.1	1.93	7,390	101.0	3.7	15,060	221	8.05	12,681	7.80	219	139.7	104.0	17.5	7.80	219	139.7	104.0	17.5	808			
10 33-M	65.5	1.79	39.3	2,802	42.8	1.56	4,618	70.5	2.6	14,850	177	6.90	11,904	6.96	266	144.0	103.3	18.5	6.96	266	144.0	103.3	18.5	1,639			
11 45-M	68.4	1.87	42.6	3,704	44.1	1.73	7,408	88.3	3.45	12,420	169	6.47		7.17				22.1	7.17								
12 51-M	84.0	2.15	50.0								12,970	163	6.35														
13 42-M	73.7	1.92									11,960	195	6.92	10,016	8.25	225	141.2	101.0	18.2	8.25	225	141.2	101.0	18.2	963		
14 45-F	61.40	1.72	48.6	2,721	44.3	1.57	5,290	86.2	3.1	11,960	195	6.92	10,016	8.25	225	141.2	101.0	18.2	8.25	225	141.2	101.0	18.2	963			
15 62-F	79.55	1.84	34.5	3,082	38.8	1.67	4,715	59.2	2.6	13,360	168	7.26	12,567	6.82	211	137.4	104.0	12.4	6.82	211	137.4	104.0	12.4	583			
16 62-F	61.75	1.68	44.4	2,700	43.7	1.61	4,856	78.7	2.9	10,250	166	6.10	8,585	7.42	203	134.5	110.0		7.42	203	134.5	110.0					
17 43-F	69.83	1.80	43.7	2,773	39.7	1.54	4,923	70.5	2.7	13,360	191	7.42	12,023	8.03	222	137.5	106.0	18.1	8.03	222	137.5	106.0	18.1	893			
18 26-F	60.62	1.37	49.3	2,368	39.1	1.73	4,670	76.9	3.4	11,430	188	8.35	9,818	8.29	196	134.0	99.5	20.7	8.29	196	134.0	99.5	20.7	966			
19 38-F	59.50	1.59	40.3	3,163	52.2	1.95	5,194	87.2	3.3	10,260	172	6.45	8,671	6.59	205	134.5	104.0	17.0	6.59	205	134.5	104.0	17.0	884			
20 62-F	67.70	1.74	41.8	2,658	39.2	1.54	4,560	67.3	2.6	11,740	173	6.83	10,628	7.85	208	137.6	104.6	18.3	7.85	208	137.6	104.6	18.3	835			
21 35-F	60.49	1.60	42.8	3,110	49.8	1.64	5,440	89.9	3.4	12,130	201	7.53	10,460	8.16	254	136.2	102.5	16.3	8.16	254	136.2	102.5	16.3	887			
22 17-F	52.27	1.53	36.8	2,598	49.7	1.69	4,410	78.6	2.7	11,430	219	7.42	10,583	7.47	194	136.5	102.6	15.3	7.47	194	136.5	102.6	15.3	659			
23 39-F	62.13	1.62	41.7	2,857	45.9	1.76	4,870	78.7	3.0	11,430	219	7.42	10,583	7.47	194	136.5	102.6	15.3	7.47	194	136.5	102.6	15.3	842			
24 20-F	54.01	1.51	37.5	2,736	50.6	1.81	4,378	81.0	2.9	11,810	218	7.82	10,879	7.66	217	137.6	104.4	17.2	7.66	217	137.6	104.4	17.2	714			
25 40-F	68.78	1.76	43.8	3,421	49.8	1.95	6,080	88.4	3.5	11,810	218	7.82	10,879	7.66	217	137.6	104.4	17.2	7.66	217	137.6	104.4	17.2	714			
26 53-F	69.51	1.76	44.3	2,518	49.8	1.95	6,080	88.4	3.5	11,810	218	7.82	10,879	7.66	217	137.6	104.4	17.2	7.66	217	137.6	104.4	17.2	714			
27 48-F	68.4	1.71	49.3	2,518	37.2	1.49	5,003	73.7	2.9	11,540	166	6.56	10,424	7.31	234	139.6	106.4	19.1	7.31	234	139.6	106.4	19.1	1,161			
28 20-F	47.8	1.42	46.5	2,254	46.2	1.58	4,215	89.3	3.0	11,270	165	6.58	9,438	6.88	176	143.0	105.6	21.3	6.88	176	143.0	105.6	21.3	1,070			
29 10-F	66.7	1.57	42.5	2,526	38.5	1.63	5,375	80.7	3.4	8,980	184	6.33	7,490	7.65	172	140.5	101.5	19.5	7.65	172	140.5	101.5	19.5	821			
30 30-F	62.0	1.90	42.4	2,526	42.5	1.85	6,146	74.2	3.2	10,090	165	7.00	8,731	8.08	206	139.7	99.5	19.8	8.08	206	139.7	99.5	19.8	1,063			
31 44-F	53.2	1.51	41.7	2,563	48.2	1.70	4,490	82.6	2.9	14,180	171	7.19	12,350	7.80	274	143.3	105.6	16.9	7.80	274	143.3	105.6	16.9	1,075			
32 24-F	67.01	1.54	41.4	2,554	49.3	1.61	4,025	73.3	2.9	12,370	188	7.74	11,070	7.33	173	140.1	100.0	17.8	7.33	173	140.1	100.0	17.8	782			

meters and in relation to weight and surface area. Data headed extracellular fluid volume (formula A) are calculated by the original formula of Crandall and Anderson.¹ In the column headed extracellular fluid volume (formula B), the formula of Laviètes and others is used, and the values are lower inasmuch as water of red blood cells is deducted. This difference is partially offset, however, by the correction the latter formula makes for binding of thiocyanate by serum protein. Hematocrit readings were made by adding 4 c.c. of blood to 1 c.c. of 1.1 per cent sodium oxalate solution and centrifuging in hematocrit tubes until no further change in the reading occurred, precautions being taken against loss of carbon dioxide. Serum sodium was determined by the gravimetric method of Butler and Tuthill,⁵ serum chloride by the method of Wilson and Ball,⁶ total nitrogen of plasma by the macro-Kjeldahl method.⁷ Oxygen capacity was determined, using heparinized venous blood drawn without stasis and equilibrated with room air at room temperature.⁸

SUMMARY

1. Modifications are described in the method of determining extracellular fluid volume by the thiocyanate technique.
2. Body fluid analyses in 33 normal individuals are reported.

REFERENCES

1. Crandall, L. A., and Anderson, M. N.: Estimation of the State of Hydration of the Body by the Amount of Water Available for the Solution of Sodium Thiocyanate, *Am. J. Digest. Dis. & Nutrition* 1: 126, 1934.
2. Laviètes, J. B., Bourdillon, J., and Klinghoffer, K. A.: The Volume of the Extracellular Fluids of the Body, *J. Clin. Investigation* 15: 261, 1936.
3. Gregersen, M. I., and Stewart, J. D.: Simultaneous Determination of the Plasma Volume With T-1824 and the "Available Fluid" Volume With Sodium Thiocyanate, *Am. J. Physiol.* 125: 142, 1939.
4. Gregersen, M. I., Gibson, J. J., and Stead, E. A.: Plasma Volume Determination With Dyes: Errors in Colorimetry; the Use of the Blue Dye, T-1824, *Am. J. Physiol.* 113: 54, 1935.
5. Butler, A. M., and Tuthill, Elizabeth: An Application of the Uranyl Zinc Acetate Method for Determination of Sodium in Biological Material, *J. Biol. Chem.* 93: 171, 1931.
6. Wilson, D. W., and Ball, E. G.: A Study of the Estimation of Chloride in Blood and Serum, *J. Biol. Chem.* 79: 221, 1928.
7. Wong, S. Y.: The Use of Persulphate in the Estimation of Nitrogen by the Arnold-Gunniag Modification of Kjeldahl's Method, *J. Biol. Chem.* 55: 427, 1923.
8. Van Slyke, D. D., and Neill, J. M.: The Determination of Gases in Blood and Other Solutions by Vacuum Extraction and Manometric Measurement, *J. Biol. Chem.* 61: 523, 1924.

MEDICAL ILLUSTRATION

SHARPNESS CONSIDERATIONS IN MAKING SCIENTIFIC ILLUSTRATIONS*

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NO ONE will doubt the value of sharp photographs when used for scientific purposes. The very reason for using photography in illustrating case records or investigations dictates that sharpness should be a prime requisite, because the oddity of many studies calls for photographic illustrations in order to convey properly the full meaning of the problems or disclosures at hand. There is no better way to present a new situation than by photographic means, but so often we find that pictures of immense value to the individual investigator are of no value to other workers and students. By this, I mean that an investigator intimately concerned and entirely familiar with a given undertaking is able to see in a picture the real meaning it has in connection with some particular point, while to a more or less neutral or uninformed worker the picture will not have the same significance unless it clearly depicts the situation under observation. To do this the image must be sharp, and the sharper the better.

Of course, there are several considerations to be taken into account when attempting to achieve a high degree of perfection in photographic illustrating. The proper films must be chosen, the lighting arrangement on the subject must be correct, proper exposure and development should be given, and many other fundamentals should be executed exactly. Most of these requirements are topics within themselves. However, our interest in them at this time will be limited to the extent of the effect that these might have upon the subject of sharpness. They will be discussed as incidental matters at the points where they appear in the discussion at hand.

To define sharpness is not as easy as one might imagine. In general, we all have a common understanding of what is meant by the term. But other visual factors come into play that may cause confusion of the real issue. For example, from a given negative two prints can be made—one on soft paper and the other on hard. To an untrained observer and to many experienced people, the print on hard paper will always be interpreted as the sharper, whereas actually it can easily differ only in the degree of contrast. This same confusion is experienced to a less pronounced degree by viewing prints under different levels of illumination, where the variations in brightness result in a change in visual acuity.

I should not care to take the stand that *effective* visual sharpness cannot be altered by such means. On the contrary, it has always seemed to me that the

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visual definition of a photograph is intimately bound with the *ease* of ready interpretation. On the other hand, from a more critical standpoint, ease of interpretation (or readability) and actual definition are distinct matters. I shall have occasion to refer to this later.

In the practical production of sharp photographs there are several distinct and unquestionable factors. First, there is the subject itself. It will be agreed that the ultimate goal is to record all of the detail in the original subject and often, if possible, to exaggerate the ease of recognizing this detail by pure photographic possibilities, for example, exaggeration by contrast increase, bettering tone separation by filters and stains and other methods. From a practical standpoint the subject matter must be observed carefully because a working tolerance exists that in some cases permits workmanship of considerable mediocrity, while in others the tolerance is so small that exacting requirements must be complied with at every step. Probably this point deserves exemplification. Imagine that a department for years has been required to photograph gross objects having only massive details. For this work a lens and camera of average quality, adjusted quickly by unaided vision, would perhaps be found entirely adequate. Now suppose the same department is called upon to supply 8 by 10 photographs of some small, highly detailed sections of gross objects. The same equipment and methods of working in this case, as used for the mass-detailed gross objects, would certainly be found lacking in the capacity to produce sharp results. For this reason it is a good idea when making photographs of any type to attempt to procure the maximum sharpness at all times. This may require the use of reference objects possessing fine details in many cases, but the need for such simple accessories cannot be considered as a barrier to the general adoption of the principle of maximum sharpness at all times.

In arranging the lights before making a picture one should profit by the experience gained in the use of the microscope. In microscopy we are concerned with subjects of exceptionally fine details. It is well known that the finest details, especially when the magnification limit of the instrument is employed, are readily lost by a slight shift from the optimum direction of the principal light beam. To be sure, when making photographs of gross objects the placement of the light source is not so critical as in high-power microscopy, but the fact remains that when fine details within the mass areas of a gross object must be recorded, the judicious use of lights is important. It is observed by most prolific workers that diffuse light sources, although probably giving slightly less than optimum results, will permit more tolerance in placement than specular (condensed or directionally reflected) sources. However, in both cases it is usually true that the lights should not be placed so close as to produce glare and should be at average angles between the horizontal and perpendicular positions, that is, around 45 degrees.

Perhaps the most important equipment consideration is the lens of the camera. It is universally recognized that there exists a wide variation between different types and makes of photographic objectives as far as sharpness is concerned. It is further known that in practice the wide aperture lenses (of a given focal length) are usually less capable of producing a sharp image over a wide field than lenses of smaller aperture. Theoretically, the reverse is true,

for the theoretical resolving power of an objective increases with an increase in numerical aperture. However, lens makers find that a sacrifice is usually made in sharpness when the aperture is very large. At the same time, when the aperture is very small sharpness again is lost, owing to the introduction of diffraction. Apart from this generalization, objectives of poor quality most frequently are the low price types in which insufficient correction has been introduced either in the design or in the grinding of the components. A lens of good make is generally expensive, but because a lens is expensive does not give assurance that it will be of the highest possible sharpness. The best practice is to select a lens from a reputable manufacturer's catalog on a trial basis. If the lens is found to perform the duties required in one's work, nothing more can be asked. If it does not comply with one's requirements, another lens can be tried. At this point it should be said that lenses of good make are in most cases capable of giving better definition than one will find possible to utilize in the photographic process. The limit of film resolution is much lower than the best lens resolution, and even the average good lens has better resolving power characteristics than the average negative film.

In spite of the fact that good lenses exceed the resolving power of most negative materials, a lack of sharpness is observed readily in much of the photographic work produced in biological departments. There must be reasons for this beyond the equipment problem, the chief one being the improper use of the equipment, especially in focusing the camera image.

Focusing is accomplished habitually by simple, unaided visual means in most departments. That this is a bad practice when optimum sharpness is imperative cannot be stressed too emphatically. When ground-glass focusing cameras are employed, it is often advisable to rub the ground glass with vaseline in order to reduce the tendency of the coarse texture to break up the image detail. Focusing should then be done with a magnifying glass of sufficient power to enable clear distinction of the finest details. At every step the perfection of focusing should be checked. If a camera without a focusing ground glass is used, the focus should be checked by placing a section of ground glass in the film plane and examining the image with a magnifier. This is all-important since print sharpness is directly dependent upon negative sharpness.

Upon procuring critical sharpness in the image plane by the proper selection and use of equipment, the next step is to record the image on film with as much fidelity as possible. Naturally, films of maximum ability to record fine details, or those having the highest resolving powers, are ideal and should be selected when other factors permit. By other factors is meant emulsion speed requirements, color sensitivity, and so on.

The resolving power of a film emulsion is difficult to express exactly, since considerable variation exists in its evaluation by different observers. Skilled workers can determine resolving power within 10 per cent of each other, whereas those who are unskilled have difficulty in arriving at results within 30 per cent variations. As a result the resolving powers of Agfa emulsions are arranged in groups, each group differing from the next by about 25 per cent. An emulsion listed in any group will have a resolving power within the limits for the

group, but some films will have slightly better resolving power than others of the same class. The general classification as it now stands is given below:

40 to 50 Lines per mm.

Ultra-Speed Panchromatic, 35 mm.

Superpan Reversible, 35 mm.

Standard

Portrait

S. S. Plenachrome

Superpan Portrait

Triple S Pan

S. S. Panchromatic

Commercial Pan

Triple S Pan Aero

50 to 65 Lines per mm.

F. G. Plenachrome, 35 mm.

Plenachrome Roll

Super Plenachrome Roll & Pack

Finopan, 35 mm. & Roll

Superpan Supreme,

35 mm., Roll & Pack

Infra Red, 35 mm.

Superpan Press, Roll & Sheet

Commercial

Commercial Ortho

Super Plenachrome Press

Isopan

S. S. Panchromatic Aero

16 mm. Plenachrome Reversible

16 mm. Panchromatic Reversible

Twin 8 & 16 mm. Hypan Reversible

16 mm. Triple S Superpan Reversible

Direct Duplicating

Direct Copy

65 to 85 Lines per mm.

Positive, 35 mm.

Process

8 mm. Filmopan Reversible

85 to 105 Lines per mm.

Printon

105 to 150 Lines per mm.

Reproolith

Reproolith Ortho

The resolving power of an emulsion is a direct expression of its ability to register fine detail. In order for the complete possibilities of the emulsion to be realized in practice, however, the sharpness factors that have already been pointed out must be considered carefully, especially the focusing factor.

After a film of given laboratory determined resolving power is selected for use, it must be exposed and developed properly if good picture quality is to be expected. This fact is well known. But it is not well known that camera exposure and development, within reasonable limits, have little bearing on the sharpness of the final negative image. Of course, when light falls upon an emulsion there are many reasons to cause one to assume that exposure and development should be critical for optimum sharpness. We know that irradiation within the emulsion by reflection and refraction of the light, and halation by back reflection will promote image spread and tend to merge the details. We know further that certain developers result in coarser-grained images than others, and that developed contrast and fog level also affect clarity of definition. However, as was pointed out earlier in this discussion, a distinction must be drawn between ease of interpretation and actual definition. It has been found that exposure and development factors have little bearing on the accuracy of determining emulsion resolving power. This alone does not mean that in practice these points should be ignored. Surely it is more desirable to have a negative in which the details are well separated by clean-cut contours, and at a density and contrast level that enable ready printing. On the other hand, it is enlightening to know that it is not so important as was once thought to have critical exposure and development in order to utilize all the resolution that an emulsion possesses.

Positive paper prints are inevitably made from negatives in biological photography. In this step much sharpness can be lost unless an exacting technique is used. The two principal methods for making paper prints, that is, by contact and by projection, involve much care. For example, in making contact

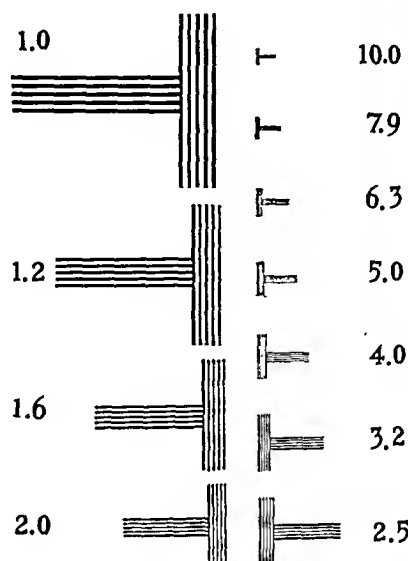


FIG. 1.—RESOLUTION CHART

The figures indicate the number of lines per millimeter in the chart. To determine the number of lines resolved by the film, multiply the corresponding number by the reduction ratio.

To aid in determining the reduction ratio, the box surrounding these instructions has been made exactly 100 millimeters in length inside dimensions.

prints we often find workers simply holding by hand, a sheet of paper against the negative on the printer top, apparently without knowing how necessary it is to get uniform and intimate contact between the developed negative and the paper emulsion. That this factor is of prime importance is borne out by the care photoengravers take in getting a good strong contact when making prints

from half-tone or line negatives. In the graphic art industry, contact printing is practically always accomplished by the use of vacuum frame printing devices. Actually, the details that are necessary in such work are of a much greater order than 50 lines per millimeter. This is the average resolving power of negative emulsions; therefore, when making contact prints from sharp negatives of biological subjects, for example, it is often necessary to get as good contrast as required in graphic art work. To accomplish this effectively and without the use of a vacuum, a strong spring contact printing frame can be employed to hold the negative and paper. This frame is held at a distance of about 6 or 8 feet from a light source approaching a point, such as an automobile headlight lamp or a clear bulb Mazda lamp. In this way an almost parallel light is used that has less tendency to spread the image detail when the shadow image of the negative is formed on the surface of sensitized paper.

In contact printing it is imperative to use glossy paper if the foregoing conditions are to mean anything. Intimate and uniform contact against a rough surface paper is indeed difficult, if not impossible.

In projection printing a more serious problem arises in attempting to retain in the enlarged print all the detail in the original negative. To begin with, the resolution of detail in a negative can easily give an appearance of absolute sharpness and yet upon projection it may break down. Such a condition is often experienced in amateur box camera photography. Visually, box camera negatives can be very sharp and can give contact prints that appear sharp. However, upon enlarging any negative image made with a simple lens, definition progressively decreases, assuming a constant print viewing distance. The real limiting factor is the acuity of the visual mechanism. It is well known that the optics of the human eye is imperfect; nevertheless, an acuity corresponding to a resolving power of about 150 lines per inch is not uncommon. When the details of a print from a given viewing distance appear fuzzy, it is obvious that the photographic sharpness limit has been exceeded. Contact prints can be well within the sharpness limitations of the eye, but unless negatives of a high order of definition are acquired and this carried through to the print, not many diameters of enlargement are permissible before the eye will be capable of detecting fuzziness. If the viewing distance is increased, a visual improvement is naturally effected from the standpoint of visual sharpness only, for an increase in detail does not accompany this improvement.

The elements of projecting negative images in order to retain as much definition as possible may be listed for convenience, since most of them are self-explanatory. They are:

1. The type of enlarger
 - a. Diffusion types give softer images
 - b. Condenser types give sharper images
2. The amount of scatter and extraneous light within the enlarger system
 - a. Small negatives should be masked off
 - b. The interior must be as nonreflecting as possible
3. The lens
 - a. Should be of high quality.
 - b. Should be free of dust and surface scum

- c. Should be stopped down to the point of greatest sharpness. This can be determined by means of a focusing tester, which is simply a negative of high contrast having clean-cut details that enable a sharp focus to be seen. This is aided further by the use of a magnifier.

In conclusion, it is evident that if optimum sharpness is to be attained, considerable care must be exercised, for fewer problems are critically concerned with equipment and materials than are dependent upon pure technique.

Erratum

On page 1158 of the April number of the JOURNAL in the article by Evan W. MeChesney, Ph.D., Kimball D. Spragne, A.B., and Irvine H. Marshall, M.D., entitled "The Effect of Sulfanilamide on Acid-Base Balance," the twelfth line should read: "In this sample the *hydroxyl*-ion concentration has increased twentyfold from the previous sample" instead of "In this sample the *hydrogen*-ion concentration has increased twentyfold from the previous sample."

DEPARTMENT OF ABSTRACTS AND REVIEWS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

LYMPHOBLASTOMA, Follicular, Baggenstoss, A. H., and Heck, F. J. Am. J. M. Sc. 200: 17, 1940.

A clinical and pathologic entity variously called "giant follicle hyperplasia of lymph nodes and spleen," "giant follicular lymphadenopathy," and "follicular lymphoblastoma" has been described in the literature in recent years with increasing frequency. The condition is characterized by an insidious onset with regional or general lymphadenopathy, splenomegaly, the absence of anemia or abnormal cells in the blood, and by the great radio-sensitivity of the lesions. The average duration of life after the appearance of the disease is four and a half years, but two patients have lived as long as seventeen years with this disease.

The characteristic histologic changes occurring early in the disease consist of an increase in the number and size of the follicles of the lymph nodes and of the Malpighian corpuscles of the spleen. There is a tendency toward fusion of the follicles, and the lymphatic sinuses are generally narrowed or obliterated.

There is a superficial resemblance between the histologic appearance of follicular lymphoblastoma and the histologic appearance of hypertrophy of the secondary centers of lymph nodes resulting from toxic or inflammatory conditions. The histologic criteria which enable the histopathologist to distinguish the two conditions are outlined.

In certain rare instances in which the histologic appearance is not typical of either follicular lymphoblastoma or inflammation, it may be wise to reserve diagnosis and merely to use a descriptive, noncommittal term, such as "follicular hypertrophy," to denote the condition. Subsequent observation and biopsy frequently will bear out the suspicion that the condition in question is an early instance of follicular lymphoblastoma.

The differential criteria listed are:

HISTOLOGIC DIFFERENCES BETWEEN FOLLICULAR LYMPHOBLASTOMA AND INFLAMMATORY HYPERPLASIA

Follicular Lymphoblastoma		Simple hyperplasia of inflammatory or toxic origin	
Follicles	{	Larger and more numerous	Smaller and less numerous
		Closely packed	Scattered
		Diffuse throughout node	Arranged around cortex in concentric rows
		Frequent in medulla	Few in medulla
		Uniformly large	Vary in size
		Tend to fuse	Discrete
Interfollicular Tissue	{	Cells densely packed	Cells scattered
		Condensation of reticulum	Loose reticulum
		Sinuses narrowed or blocked	Sinuses open, often dilated
		Slight proliferation of reticular cells	Marked proliferation of reticular cells

SPUTUM, Aspiration of, From Adults, Auger, W. J. J. A. M. A. 115: 1186, 1940.

A brief study was carried out on 13 adults with pneumonia who reputedly could not cough or bring up satisfactory sputum for examination.

In all 13 cases sufficient sputum was obtainable from either the larynx or the nasopharynx by the use of the aspiration method.

No advantage was found in nasopharyngeal secretions as compared with secretions from the larynx in the typing of pneumococci in pneumonia among adults.

The laryngeal aspiration method of collecting sputum, whether obtained by syringe and finger valve or by a sputum trap and machine suction, has been suggested for adults when voluntary efforts at expectoration have failed. This method is also recommended for obtaining sputum from tuberculous patients.

It is suggested that the person who examines the sputum in the laboratory should obtain the sputum.

BRUCELLA, An Evaluation of the Opsonocytophagic Test, Wise, B. Am. J. M. Sc. 200: 520, 1940.

The usual range of the Brucella opsonocytophagic index in normal individuals, as expressed by the index number, is from 0 to 30.

A positive Brucella opsonocytophagic index is specific, that is, the individual's blood that is positive for Brucella does not phagocyte other organisms to any considerable degree.

A significant difference in the phagocytosis of nonencapsulated and encapsulated strains of Brucella organisms exists, and should be considered when this test is carried out.

The Brucella opsonocytophagic index has its greatest usefulness in indicating contact with Brucella organisms and, of the usual laboratory tests, is the single most useful test for survey work.

The Brucella opsonocytophagic index is not diagnostic of active Brucella infection and may be negative when active infection exists.

TUBERCLE BACILLI, Demonstration of, Steenken, Wm., Jr. Am. Rev. Tuberc. 42: 548, 1940.

An alcohol-precipitated protein from pleural fluid and white of egg has been recommended for sedimenting tubercle bacilli from urine and spinal fluid in place of tannic acid.

The sedimented protein is soluble in 4.0 per cent sodium hydroxide. It gives a straw color which does not interfere with color change of the indicator used for adjusting the pH.

The protein is not inhibitory to the growth of the tubercle bacillus as the tannates are.

The method follows:

Preparation of protein: All glassware used in the preparation of the protein must stand in cleaning solution (mixture of sulfuric acid and potassium dichromate) overnight.

Clear colored pleural fluid, filtered through paper and centrifuged at high speed for a half hour, was diluted with an equal volume of distilled water and passed through an "N" Berkefeld filter. This filtrate was sterilized at 60° C. for one hour, and the protein was precipitated by the addition of three volumes of 95 per cent alcohol for every volume of pleural fluid. The resulting mixture of precipitated protein and alcohol was allowed to stand in the icebox overnight, at the end of which time the alcohol was decanted and the remaining protein washed three times with equal volumes of 95 per cent alcohol. Finally it was centrifuged and the resulting sediment was suspended in 50 per cent alcohol, then it was diluted so that each cubic centimeter contained 50 mg. dry weight of the protein. It was then sterilized at 50° C. for one hour and stored in the icebox until ready for use.

The protein may also be prepared from the white of egg using the same procedure, only it is recommended that the eggs be fresh and that the whites be dissolved in an equal volume of water before precipitating with the alcohol.

Treatment of specimen: A twenty-four-hour specimen of urine is collected in the usual manner, and for every 100 c.c. of urine, 1 c.c. of the protein suspension is added. The resulting mixture of protein and urine is allowed to stand in the icebox for twenty-four

hours. At the end of this time most of the protein will have sedimented to the bottom of the bottle carrying the tubercle bacilli with it. The supernatant urine is then siphoned off and the sediment is centrifuged at high speed for ten minutes to free it of the residual urine. The sediment is taken up with an equal volume of 4 per cent sodium hydroxide and placed in the incubator for half an hour. The digested material is then centrifuged at high speed and the supernatant fluid is decanted. The sediment is neutralized to pH 7.0 with tenth-normal hydrochloric acid containing phenol red, when it is ready for smear culture or animal inoculation.

Tubercle bacilli may be recovered from spinal fluid by the addition of 0.1 c.c. of protein for every 10 c.c. of spinal fluid. The mixture is then centrifuged at high speed. The sediment is now ready for smear culture or animal inoculation.

Fluids of higher specific gravity that are miscible with water may be sedimented if diluted with sterile distilled water. Other alcohol-precipitated proteins may also act as sedimenting agents.

The criticism may arise that the pleural fluids used to prepare the protein may have been contaminated with a few tubercle bacilli, but such organisms would be dead. The necessary check of all positive urine or spinal fluid smears by culture of guinea pig inoculation would remove this objection.

COVER SLIPS, A Practical Substitute for, Norris, J. C. J. A. M. A. 115: 1099, 1940.

Norris recommends plastacele securable in sheets (C-12-7511-sheet 20 by 50 inches by the E. I. DuPont Co., 626 Schuyler Avenue, Arlington, N. J., at \$1.00 per sheet). One sheet can be cut into about 1,000 cover slips.

TUBERCULIN REACTION, The Instability of, Dahlstrom, A. W. Am. Rev. Tuberc. 42: 471, 1940.

The dispensary records of 3,919 members of 513 families under observation from five to fifteen years were examined to determine whether the tuberculin reaction, once established, remains constant, and, if not, to determine the circumstances of inconsistency, with particular reference to the original intensity of the reaction, the degree of exposure to tuberculosis, the presence of tuberculous lesions, race, age, and sex, and finally the season of the year.

Of the group examined, 2,490 were positive to tuberculin in at least one examination, and 276, or 11.1 per cent of these, passed from the positive to the negative state during the period of observation in the dispensary. The lower the original degree of sensitiveness, the greater was the likelihood of reversal from positive to negative reaction. Only 0.4 per cent of 1,090 people giving a three-plus reaction to the standard first dose of tuberculin became tuberculin-negative, whereas the negative state supervened in 70 per cent of 185 persons, giving only a one-plus reaction to the second dose.

The greater the degree of family exposure, the more likely a reaction was to remain positive. In families with no history of tuberculosis in any member, 24 per cent of the members became negative, whereas among 63 reactors in families in which a patient with positive sputum resided constantly, only one person (1.6 per cent) became negative.

The tuberculin reaction never became negative in the group under consideration in the presence of lesions of active reinfection type tuberculosis, but became negative in a small number of cases of tuberculous lesions of the type diagnosed by x-ray examination only. The list included 2 cases diagnosed as active childhood type tuberculosis, 5 cases with inactive or suspected reinfection type tuberculosis, and 11 cases with x-ray evidence of calcified childhood tuberculosis. Altogether, reactive capacity disappeared in 0.72 per cent of the total number of patients with recognizable tuberculous lesions of any sort.

No significant correlation of instability of the tuberculin reaction with sex was discovered, but there was definite correlation with age. The overwhelming majority of the unstable reactors were children. Reversion to negative reaction proved rare in adult life. Race possibly played a part, but uncontrollable variables made definite determination impossible. When comparison was made of two largest racial groups, Italians and Negroes, the former appeared to react less intensely and to have more tendency to lose a sensitivity once it was conferred.

GRANULOMA, Systemic Reticulo-endothelial, Wallgren, A. *Am. J. Dis. Child.* 60: 471, 1940.

A description is given of two clinically and anatomically investigated patients with systemic reticulo-endothelial granuloma, they being 4 months and 2½ years of age, respectively. These cases, which essentially show great similarities, occupy intermediate positions, one of them between so-called infectious reticulo-endotheliosis and Letterer-Siwe disease, and the other between the latter malady and Schüller-Christian disease.

These three types of systemic reticulo-endotheliosis, hitherto regarded as different diseases, are discussed, and reasons are advanced to show that the distinguishing features between the two first mentioned are rather arbitrary and nonessential, and that the anatomic and clinical dissimilarities that may exist between nonlipoid reticulo-endotheliosis and Schüller-Christian disease are easily explained by the difference in age of the patients, the unequally malignant or benign course, mainly due to this difference in age, and the different localization of the morbid changes. Transitional types are found between characteristic cases of Schüller-Christian disease and Letterer-Siwe disease and also between those of the latter and so-called infectious reticuloendotheliosis.

ANEMIA, "Target-Cell," Dameshek, W. *Am. J. M. Sc.* 200: 445, 1940.

In an Italian youth with hypochromic anemia, splenomegaly, and a hemolytic type of icterus, unusual changes were discovered in the red blood cells and the bones. About a third of the red blood cells presented the appearance of "targets" or "bull's eyes"; these cells were unusually resistant to hypotonic solutions of sodium chloride. No nucleated red blood cells were present. Generalized osteoporotic changes with great thickening of the skull were present; in addition, a bony tumor arising from a rib encroached on the right upper lung. Target cells have been described in sickle-cell anemia, cirrhosis of the liver, chronic hypochromic anemia after splenectomy, and in other conditions. The present studies have shown that they are also common in Cooley's erythroblastic anemia.

The condition of "target-cell anemia," as described in this paper, may be a formes frustes, an anerythroblastic type of Cooley's erythroblastic anemia or a closely related condition. The possibility is broached that Cooley's anemia, sickle-cell anemia, and target-cell anemia are related conditions with target cells and increased saline resistance as common denominators. In Cooley's anemia the target cell is probably a more fundamental abnormality than the erythroblast, and may represent the basic hereditary defect. In these atypical hemolytic states, a lack of response to splenectomy further differentiates them from most cases of congenital and acquired hemolytic icterus.

Note: This condition has also been described by Wintrobe, Mathews, Pollack, and Dobyns (*J. A. M. A.* 114: 1530, 1940).

HYPERTENSION, Orthostatic, McCann, W. S. *J. A. M. A.* 115: 573, 1940.

In some instances of nephroptosis the erect posture may result in orthostatic elevation of the blood pressure, with diminution of the total renal blood flow and with relative consistency of glomerular filtration, though the "filtration fraction" is increased. The clearance ratio of diodrast to inulin is decreased in erect posture as compared with that in recumbent posture.

Patients with normal blood pressure and those with hypertension but without nephroptosis did not exhibit these changes.

Hypertensive patients should be studied with regard to the effect of posture on the blood pressure by comparing the pressure during ordinary activity with that found after a period of strict recumbency. Those in whom a marked orthostatic effect is demonstrated should receive pyelographic study and treatment by appropriate support of the kidneys which are ptosed. Cerebral, ocular, and cardiac symptoms may be relieved by the stabilization of blood pressure which results from such measures.

TOBACCO, Cutaneous Manifestation From, Barksdale, E. E. J. A. M. A. 115: 672, 1940.

It has been proved by other workers and the author that tobacco contains arsenic. It is common knowledge to those living in a tobacco section that farmers freely use lead arsenate as an insecticide to kill the corn worm. Nothing has been done or probably can be done in either the agricultural or manufacturing process to remove it. A series of cases with exfoliative dermatitis and positive blood arsenic, whose sources of arsenic were thought to be tobacco, was observed. The blood in a series of cases of severe dermatoses and normal individuals has been analyzed for arsenic and found to be negative, even though the patients were heavy smokers. It is thought that there is a sufficient amount of arsenic in tobacco to have a very detrimental effect in cases of postarsphenamine dermatitis.

It is realized that the Gutzeit test may not be sensitive enough to determine a difference in the blood arsenic of smokers and nonsmokers.

It is believed that patients get this dermatitis because they are hypersensitive or allergic to arsenic.

This paper has attempted to present a theory. Its ultimate proof depends on the collaboration of other workers.

SULFAPYRIDINE, Pathologic Changes Following Prolonged Administration of Sulfathiazole and, Rake, G., van Dyke, H. B., and Corwin, W. C. Am. J. M. Sc. 200: 353, 1940.

Sulfathiazole when given as 2 per cent of the diet killed 77 per cent of mice during a four-week period and produced lesions chiefly in the spleen and genitourinary tract. Sulfapyridine was not lethal and produced fewer pathologic changes.

In rats sulfapyridine was twice as toxic as sulfathiazole as shown both by the effect on the growth curve and by the lesions produced in the genitourinary tract.

In monkeys receiving a single daily dose sulfapyridine was more toxic than sulfathiazole, as shown by the lesions in the genitourinary tract and, to a lesser extent, by loss of weight and leucopenia.

LEUKOCYTES, Motility and Chemotaxis of, in Health and Disease, Mallery, O. T., Jr., and McCutcheon, M. Am. J. M. Sc. 200: 394, 1940.

The activity of polymorphonuclear leucocytes from a series of patients acutely ill, most of them with infections, was compared in vitro with that of the observer's leucocytes and of leucocytes from patients not acutely ill. Decreased rate of locomotion and less direct approach to bacteria were shown by leucocytes of the acutely ill persons. In leucocytes of some patients who were gravely ill decline in motility and chemotactic response was progressive, whereas the observer's leucocytes maintained their activity unimpaired for hours. The functional changes observed would tend toward lowering resistance to infection. These experiments emphasize the fact that in severe illness the function not merely of whole organs but of individual cells is depressed, and they afford a method for quantitative measurement of changes in cellular function.

Books and Monographs for Review should be sent direct to the Editor,
Dr. Warren T. Vaughan, 201 West Franklin Street, Richmond, Va.

WELL written, easy to read, this book is short, concise, and well translated. The author takes up the various anorectal conditions one by one, discussing the etiology, pathology, and treatment of each. The sections on etiology present a new concept which is of interest. It is brought out that the underlying cause of hemorrhoids, anal fissure, fistula in ano, pruritus ani, and rectal prolapse is a congestion of the portal circulation. The author maintains that all are merely symptoms of the same condition. He further points out that all respond to the treatment, namely, injection of a sclerosing solution around the hemorrhoidal veins. A chapter is included on the instruments necessary for injection treatment.

The book contains many well-reproduced color photographs and drawings in addition to clear-cut black and white illustrations.

ALTHOUGH the first edition of this volume appeared as recently as 1936, it has been necessary to put out a new edition every two years, this being the third. This volume has several unusual features. It contains practically fifteen hundred pages. It is more abundantly illustrated than any other textbook on the practice of medicine, there being 562 illustrations, 48 of them in color. This is the first medical text using the new green tinted paper which reduces glare and eyestrain. The discussion of individual diseases is broader than in other similar texts in that discussion includes illustrations which are usually found in texts on physical diagnosis and clinical pathology. In other words, the discussion is broader. At the same time there is no superfluous verbiage, the textual presentation being very succinct. This volume may be highly recommended.

SINCE short wave therapy is based on physical, technical, and medical principles, it is fortunate that collaboration of a physicist and a physician led to the production of this book. A short introduction to the physical theory of oscillations is given, followed by the most important methods of measurement for technicians and doctors. Important considerations about the choice of apparatus are mentioned. The medical section contains a review of the

*Haemorrhoids and Their Treatment: The Varicose Syndrome of the Rectum. By Kasper Blond, M.D., Vienna, Formerly First Assistant, Rothschild Hospital, Vienna; Hon. Consulting Surgeon, Municipal Hospital, Vienna. Translated by E. Stanley Lee, M.S., F.R.C.S., Hon. Assistant Surgeon, Westminster Hospital. Cloth, 140 pages, \$4.50. A William Wood Book. The Williams & Wilkins Co., Baltimore, 1940.

†The Practice of Medicine. By Jonathan Campbell Meakins, M.D., LL.D., Professor of Medicine and Director of the Department of Medicine, Royal Victoria Hospital, Montreal; Formerly Professor of Medicine, University of Edinburgh; Fellow of the Royal Society of Medicine, London; Fellow of the Royal College of Physicians, Edinburgh; Fellow of the Royal College of Physicians, London. Cloth, ed. 3, 1430 pages, with 562 illustrations, including 48 in color. The C. V. Mosby Company, St. Louis, 1940.

American College of Physicians. Cloth, ed. 3, 1430 pages, with 562 illustrations in color. The C. V. Mosby Company, St. Louis, 1940.
 ‡Foundations of Short Wave Therapy. Physics—Technics—Indications. An Introduction to the Physico-Technical Principles and Medical Applications of Short Electric Waves for Physicians and Biologists. Physics and Technics. By Wolfgang Holzer, Dr. Ing., Assistant in the Physiological Institute of the University of Vienna. Medical Applications. By Eugen Weissenberg, Dr. Med., Medical Superintendent of the Short Wave Section of the University Clinic for Nervous and Mental Diseases in Vienna. Translated by Justina Wilson, F.R.C.P. Edin., D.M.R.E. Cantab., and Charles M. Dowse, B.Sc. Eng. Lond., A.M.I.E.E. Cloth, 228 pages, with 53 illustrations and 10 tables, \$5.00. Distributed by Chemical Publishing Company, Inc., 148 Lafayette St., New York.

whole field of indications for short waves. Diseases of the skin, such as varicose eczema and localized suppurating inflammations, inflammations of the soft tissues, and diseases of bones and joints are particularly amenable to short wave therapy. It is remarked that gonococcal arthritis reacts especially well. Diseases of the lungs, including asthma, are also said to respond. It would appear that there is no diseased organ system that cannot be benefited to a greater or lesser extent by this technique. The book is ably translated and contains a very complete bibliography. It is felt that this is an excellent presentation and will be of equal value to clinicians and experimental workers.

Clinical Parasitology*

THE second edition of this book is most complete and extremely well illustrated.

While older books on human parasitology have classified parasites on the basis of their location or the manner of their activity in the human body, the classification in this volume is in accordance with the international code of zoological nomenclature. This same trend toward more scientific classification is seen in bacteriology, mycology, and allergy. It represents a natural scientific evolution and facilitates precision in discussion.

The volume goes farther than the older writings on human parasites, including not only protozoa and helminths, but also the arthropods, spiders, lice, bees, flies, fleas, and similar animal parasites or enemies.

A technical appendix provides ample instruction for laboratory study.

Histological Technic†

AS STATED by the author in his preface, the major purpose of this work is to present modifications of methods of tissue fixation, section-making, and staining which in his experience make the results more quickly available to the pathologist. Consistent with this aim, Krajian not only presents the generally accepted methods but also seventeen of his own modifications.

The book is well illustrated, reasonably small, and easy on the eyes because of the new type paper with a slightly greenish tint that reduces glare. It should become a useful and valuable laboratory handbook.

Bacillary and Rickettsial Infections. Acute and Chronic‡

THIS volume has a really new approach. Although history, epidemiology, prevention, symptomatology, diagnosis, and treatment are included under the various diseases, the history of the development of our present understanding is given major prominence. The volume is one which may be read for pleasure when one is relaxed and desires to broaden the horizon of his understanding of the problems of medicine as they have been solved throughout the centuries.

Marginal notations appearing as inserts are abundant and facilitate immediate orientation for those who may wish to refer to some specific phases of the discussion.

As indicated in the title, the coverage is of bacillary and rickettsial diseases, and does not include virus diseases or other forms of bacterial infection.

*Clinical Parasitology. By Charles Franklin Craig, M.D., M.A. (Hon.), F.A.C.S., F.A.C.P., Col. U. S. Army (Retired), D.S.M., Emeritus Professor of Tropical Medicine in the Tulane University of Louisiana, New Orleans, La.; and Ernest Carroll Faust, M.A., Ph.D., Professor of Parasitology in the Department of Tropical Medicine, Tulane University of Louisiana, New Orleans, La. Cloth, ed. 2, thoroughly revised, with 244 engravings, 772 pages, \$8.50. Lea & Febiger, Philadelphia, 1940.

†Histological Technic. Including a Discussion of Botanical Microtechnic. By Aram A. Krajian, Department of Pathology, Los Angeles County General Hospital, Los Angeles, Calif. Cloth, 272 pages, with 44 text illustrations and 7 color plates. The C. V. Mosby Company, St. Louis, 1940.

‡Bacillary and Rickettsial Infections. Acute and Chronic. A Textbook. Black Death to White Plague. By William H. Holmes, Professor of Medicine, Northwestern University Medical School, Chairman, Department of Medicine, Passavant Memorial Hospital, Chicago. Cloth, 675 pages, \$6.00. The Macmillan Company, New York, 1940.

The Biological Reaction*

THE reader will be astonished to find that despite this general title the authors are only discussing tuberculosis. Of course, they approach their subject from a general point of view. It is obvious that for them tuberculosis, as the disease with which they both are most familiar, serves only as an example to demonstrate how the complexity of the various factors involved in the biological reaction of the organism against a disease can be analyzed. The results of their analysis of the reaction of tuberculosis patients and animals, if correctly interpreted, are highly important for the treatment and prophylaxis of this disease. In the first part of the book, Hofflin (Victoria, Canada) gives the theoretical arguments and the mathematical proofs for their method of expressing the "biological reaction" in figures. A series of six to ten differential leucocyte counts (classification of Schilling) are made at intervals of two hours in order to interpret the oscillation of the biological reaction. The minimal and maximal differences of the various counts with one another, and the deviations of their average values from the standard values are the basis for the calculation of figures expressing seven variables of the biological reaction: extensity of foci, intensity of foci, allergy, general resistance, specific resistance, index of immunity, and metabolic index. These figures are then analyzed by means of a Fournier series and their correlations are represented geometrically in graphs with logarithmic scales (secondary analysis). To complete the functional analysis, two series of differential counts are made on two consecutive days, one before, the second after, an intracutaneous tuberculin injection. Both their graphical representations are compared and interpreted concerning their relative position to one another according to Hofflin's experience with cattle tuberculosis. In the second part of the book, Bueher (Zürich, Switzerland) reports the clinical proofs and the practical application of the new method for the diagnosis and prognosis of human tuberculosis. The third part of the book is entitled "Quantum Diagnostic. Foundations of a Modern Tuberculosis Therapy and Prophylaxis. Practical and Theoretical Conclusions." An "active" gold preparation (erysatox, described as a gold-sodium-sulfur combination) combined with a "biological carrier" (ompotan, the chemical nature of which the authors do not even intimate) is given intravenously or orally. Not only good clinical results are reported, but the new diagnostic method of the authors gives the possibility of finding out the favorable moment at which the therapy has to start, and of demonstrating later on the improvement in the biological reaction of the patients. While this therapy is declared to be a specific one, the prophylaxis proposed and tried with success by the authors on animals and patients has as its aim to raise the non-specific immunity without producing allergic reactions as proved by their new "quantum diagnostic." The prophylaxis consists in feeding a complex emulsion of large molecular lipoids, proteins, and polysaccharides, of which no further descriptions are given.

Without doubt the authors discuss many very interesting and important observations. However, only few readers will be able to follow their train of thoughts and understand and accept their conclusions. Unfortunately, the mathematical part is kept so brief that it will be difficult for the average physician to understand it, and especially to realize why one has to assume that the various proposed formulas can express allergy, immunity, resistance, etc. The experimental and clinical evidences offered are not convincing enough to vindicate such comprehensive theories.

—Ernst Fischer, M.D.

*The Biological Reaction. A functional analysis and synthesis of biometrical figures for the determination of allergy, general resistance, specific resistance, extensity of the disease, extensity of active foci, and immunity. (Die Biologische Reaktion. Eine funktionelle Analyse und Synthese biometrischer Werte zur zahlenmässigen Erfassung von: Allergie, allgemeiner Resistenz, spezifischer Resistenz, Krankheitsintensität, Extensität aktiver Herde, Immunität.) Hans Huber, Bern, 1939.

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PROGRESS

SOME RECENT ADVANCES IN DERMATOLOGIC THERAPY

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THE material for the present summary has been gathered from the literature of the last two years. Selection has been entirely arbitrary, and many contributions of undoubted value have had to be omitted. Reference is made to some purely experimental work, not because it represents a practical advance in present dermatology, but because it may be the source of future progress in therapy.

VITAMIN THERAPY

The dermatologist deals primarily with the skin, the largest and most accessible organ of the human body. No other single organ offers such scope for the study of morphology and function in both health and disease. No other organ is subject to external assault by physical, chemical, mechanical, and biological agents; and at the same time, to internal onslaught by almost all the factors causing disease in more sheltered tissues. The dermatologist must, therefore, be an alert "internist" as well as a competent "externist." In many so-called "systemic diseases" a careful study of the skin and orificial mucous membranes will offer either supportive or conclusive diagnostic evidence (criteria). The avitaminoses (or "dysvitaminoses" as we prefer to call them) are a characteristic group in this category. However, the significance of the dermatologic approach to the study and management of vitamin deficiency is twofold. In the first place, it embraces the recognition of the classic general as well as local cutaneous manifestations of the various deficiency syndromes; in the second place, it includes the administration of vitamins to produce therapeutic effects on cutaneous lesions, as well as in order to achieve systemic effects or to build general resistance.

It is our opinion that many cases of vitamin "deficiencies" are not true deficiencies, but rather abnormal conditions of demand, utilization, and transport of the respective vitamins, "dysvitaminoses" (Sulzberger¹). Moreover,

in numerous instances, it seems as though improvement takes place faster if several, and not only one, of the vitamins are administered. However, it is, of course, usually necessary to give that particular vitamin or complex which is most obviously needed in dosage much higher than that of the other vitamins.

Goodman, Kanof, Bacr, and one of us (M. B. S.)² have shown that some cases of keratosis pilaris, formes trustes of ichthyosis, lichen spinulosus with or without cicatrizing alopecia, and brittleness of the nails are benefitted by large doses of *vitamin A*.

Probably the most striking of the newer studies on the role of vitamin A in dermatology is contained in the observations of Peck, Chargin, and Sobotka³ on keratosis follicularis (Darier's disease). These investigators found that individuals with Darier's disease had a normal or increased amount of the provitamin A (carotene) in their blood serum, but a decidedly lower than normal ratio of vitamin A itself. These determinations were made by use of the photometer,⁴ with which quantitative estimations of vitamin A in blood serum are made according to the method of Carr and Price.⁴ The estimations were confirmed by the dark adaptation method of Hecht and Mandelbaum.⁵ It was further found that the dyskeratosis gradually subsided when serum vitamin A levels were raised to normal by the administration of large amounts (200,000 I.U. daily) of vitamin A concentrate, and recurred when this treatment was stopped or the dosage reduced. On the basis of this preliminary work, Peck and his collaborators believe Darier's disease to be due to an hereditary physiologic weakness in the absorption of vitamin A or in its conversion from the provitamin.

Recently, also, the dermatologic condition known as pityriasis rubra pilaris has been successfully treated by vitamin A in large doses. Brunsting and Sheard⁶ had previously found that dark adaptation tests were similar to those later reported by Peck and co-workers in Darier's disease, and improvement in the dark adaptation which followed vitamin A therapy was, to some degree, paralleled by clinical improvement in the dermatosis. In this report, attention is drawn to the impairment of dark adaptation sometimes found associated with cirrhosis of the liver.⁷ Recently, cases have been reported of the beneficial effect of vitamin B complex on pityriasis rubra pilaris.⁸ This observation may lead to the assumption that the vitamin B complex produces an improvement in liver function which in turn permits the proper utilization of vitamin A, or the conversion of provitamin A to vitamin A. The reviewers have used vitamin A with some success in cases of phrynoderma, in individuals with hypothyroid habitus, and in various forms of "dryness of the skin," of lichen pilaris, of mild ichthyosis, and of follicular keratoses; and in patients giving a history of long-continued ingestion of mineral oil or of prolonged indulgence in some dietary fad. Debilitated or elderly patients with chronic dyskeratotic disease, or those suffering with chronic ulcers often respond to large doses of vitamin A, preferably complemented by B complex.

The *vitamin B complex* is justly receiving a lion's share of attention at present. It is our belief that, with a few notable exceptions, such as pellagra and polyneuritis, the vitamin B complex components are most effectively admin-

*Manufactured by the Central Scientific Co., Chicago, Ill.

istered as injections of whole crude liver extract (not the refined or concentrated preparations). This form is cheaper and much more efficacious than the 2 to 15 units per cubic centimeter products often employed. In many cases liver injections seem to be more effective also than much higher dosage of the present known vitamin B factors given by mouth. This may be due (1) to an unknown factor present in the crude liver; or (2) to a synergistic action of the various fractions of liver when given parenterally; or (3) to an alteration in composition of the concentrated or crystalline products produced by pharmaceutical houses for oral administration; or (4) to a faulty or selective alimentary absorption of the oral medication.

Madden⁹ reports vitamin B₁ to be a valuable adjunct in the therapy of psoriasis. There is a growing literature on vitamin B₁ therapy in pink disease (acrodynia) in children.¹⁰ Its use is well known in beriberi, and in the peripheral neuropathies of pellagra, alcoholism, and pregnancy.

Riboflavin (vitamin B₂) deficiency is characterized by some combination of the following signs and symptoms: redness of the buccal surface of the lips and abnormal redness of the vermilion borders with desquamation. There is usually maceration and fissuring, and sometimes rhagades-like lesions at the commissures. Seborrhea of the naso-labial folds, alae nasi, eyelids, and ears is frequent. There may be a specific type of glossitis with a purple-red color, fissuring, and coarse granular appearance. Conjunctival, scleral, and corneal injection and vascularization may be seen. The patient complains of burning of the eyeballs, roughening of the lids, visual fatigue, and photophobia.¹¹ All these conditions are promptly remedied by specific treatment—except the visual disturbances in long-standing cases. In dermatologic practice two points are worthy of stress in connection with the recent literature on riboflavin: the first is that *ordinary perlèche*, as occurring in children, or after trauma (dentist) and as seen so commonly by skin specialists, is *not a riboflavin deficiency and does not respond to riboflavin therapy*. The second point to be made is that while many of the corneal ulcers of *rosacea keratitis* respond to vitamin B₂, the cutaneous acne and *rosacea* remain unaffected, and do not, as a rule, respond to riboflavin.

Smith, Smith, and Callaway¹² describe a skin condition in pellagrins which they term "dyssebacia." The lesions, chiefly on the face, are characterized by dryness of the skin and the presence of plugs of inspissated sebum projecting from the sebaceous follicles. Dyssebacia is readily cured by autoclaved yeast by mouth or crude liver extract by injection, but is uninfluenced by oral administration of vitamin B₁ and liver extract. Nicotinic acid is effective but requires a much longer time than yeast or crude extract of liver.

The role of *nicotinic acid* in pellagra therapy is well known, as are its supposed and reported effects in alleviating some of the disagreeable subjective effects in sulfonamide therapy. The reviewers believe it may exert some beneficial effect in some cases of lupus erythematosus, perhaps by reducing the photosensitivity in this disease. They believe also that some patients tolerate oral bismuth (sobilminol) better while taking nicotinic acid, having less tendency to stomatitis and fewer gastric upsets. King¹³ reports the disappearance of organisms of Vincent's angina in forty-eight to seventy-two hours with nicotinic acid therapy.

Vincent's angina and stomatitis are important, and are at present causing *much disability in military concentrations and defense projects*, and King's findings should merit further study.

The status of *pantothenic acid, biotin (vitamin H)*, and other factors of the vitamin B complex has not been settled. A very thought-stimulating paper by György¹⁴ discusses the possible relationship of these factors (as well as thiamine and riboflavin) to the sealy desquamative dermatoses in man. He has experimentally produced and cured in animals conditions similar to those of the "seborrheic diathesis" in man.

Spies¹⁵ points out that pantothenic acid and riboflavin are mutually complementary, and believes the former to be a dietary necessity.

Kristensen and Vendel¹⁶ claim to have effected a prompt, universal, and permanent clearing in 13 acute and 7 chronic cases of eczema treated with the vitamin B complex.

The Plummer-Vinson syndrome, characterized by anemia, dysphagia, glossitis, cheilitis, and spoon nails, is considered a precancerosis, probably related to vitamin B complex deficiency.¹⁷ Notable among other suggested uses of vitamin B complex are its use in the treatment of (1) glossitis and stomatitis accompanying sprue, pernicious anemia, and pellagra;¹⁷ (2) certain cases of neurodermatitis of the back and sides of neck and the elbow flexures;¹⁷ (3) certain cases of moniliasis;¹⁷ (4) cases of lupus erythematosus with extreme sensitivity to light;¹⁷ (5) some cases of kraurosis vulvae.¹⁷

Gross¹⁸ reports improvement in cases of kraurosis vulvae by combined administration of vitamin A, riboflavin, and liver extract. He stresses the interrelationship between hormone and vitamin therapy because some of these cases may respond to either type of treatment. He believes some of the very apparent increased effectiveness of crude liver extract may be due to biotin, although it is supposed to be left in the residue of extraction. Two other important observations are made by Gross: (1) pantothenic acid therapy response depends on much larger dosage than is found in vitamin B complex; and (2) very large dosage of thiamine chloride may cause a relative deficiency of nicotinic acid due to increased requirement, although there is no known toxicity to vitamin B complex even in huge doses.

One of us (M. B. S.), in discussing vitamins A and C,¹ mentions certain instances of quicker improvement in phrynodema (dry skins and follicular keratoses) being effected by *vitamin C* therapy than by administration of vitamin A. He also describes Sjögren's¹⁹ syndrome, which is very similar to the Plummer-Vinson disease in all its manifestations. He states that this condition, which is usually seen in women at the climacteric, is uninfluenced by iron or vitamin B complex, but is much improved by large doses of vitamin A. He also makes a point of the possible relationship between malignant epithelial changes and dysvitaminosis A.

The dermatologic aspects of vitamin C are discussed by Saunders,²⁰ who states that it exerts a regulatory action on pigment production in scurvy and in Addison's disease, and favorably influences chloasma uterinum. Subclinical scurvy is important in dermatologic diagnosis and management because it is

more common than is usually supposed; and because it may intensify the hemorrhagic tendency of any existing eruption.¹⁷

Scarborough²¹ believes the hemorrhage of vitamin C deficiency to be ecchymotic or deep in muscles and tissues in contrast to that of vitamin P deficiency, which is petechial and circumpilar. Gorrie²² has successfully treated purpura hemorrhagica following neoarsphenamine with vitamin P (hesperidin). Three cases of Henoch-Schönlein type, one of rheumatic and one of symptomatic purpura, were "cured" by Hiramatsu²³ with hesperidin. He believes, however, the effects of vitamin P therapy are enhanced by vitamin C.

At one time or another vitamin D has been reported as useful, if not specific, in almost every chronic dermatologic condition. Wise and one of us (M. B. S.) have stated that their experience with vitamin D has been disappointing in the major uses for which it has been recommended in dermatology; namely, psoriasis, acne vulgaris, and pemphigus.²⁴

The beneficial effect of vitamin E (α -tocopherol) on amyotrophic lateral sclerosis, reported by Wechsler, led Baer²⁵ to treat a case of *dermatomyositis* with this drug. The patient improved under this therapy, suffered a remission when treatment was stopped, and continued to improve with resumption of α -tocopherol intramuscularly.

The role of the *unsaturated fatty acids* as vitamins is not clear. It is known that certain of these acids, such as linolenic acid, will cure rat "acrodynia" produced by pyridoxine deficiency.¹⁴ Linolenic acid also exerts a vitamin A-like effect on nail nutrition,²⁷ although its isomer, α -eleostearic acid, is without effect.

Gross²⁸ has shown that animals fed vitamin B₆, together with curative doses of essential fatty acids, were entirely protected against vitamin B₆ deficiency, but slowly developed the skin lesions of the *filtrate-factor* deficiency and died from the lack of these factors.

SULFONAMIDES IN DERMATOLOGY

The history of the sulfonamides in dermatology is much like that of their use in general medicine. The statement is almost warranted that sulfonamides have been tried on every dermatologic entity except the frank malignancies, nevi, and atrophies. As in other fields of medicine, sulfonamide therapy may be said in many respects to be still in the experimental stages. There are, of course, certain well-recognized dermatologic indications where one of the sulfonamides may be the therapy of choice, others where there is enough established evidence of possible benefit to justify its use, and then the vast limbo of "shooting arrows in the air" and of the "last resort" cases.

It is certainly not within the scope of this paper to discuss the mode of action of the sulfonamides, but the opinion may be ventured that it is probably an effect on the oxygen metabolism of the germ itself. The reader may be referred to authoritative papers on this subject.^{29, 30} The selection of the drug itself offers somewhat of a problem, some authors reporting better results in general with *sulfapyridine* than with *sulfanilamide*.

Sulfathiazole is generally accepted as being the agent of choice in staphylococcal infections and is the drug most used in topical application, either as

powder or as the soluble sodium salt. In our own investigations we have found that sodium sulfathiazole is not a primary irritant to the skin, despite the high alkalinity (pH. 11:0). As a preliminary precaution patch tests were applied on 35 normal skins before we used sodium sulfathiazole therapeutically as wet compresses and irrigations. We have used this salt in 5 per cent concentrations in ointments, in emulsions, and as compresses, employing it in infected hemostatic (varicose) ulcers, in impetigo, in furuncles, and in carbuncles. In the latter we have used a complementary oral dosage of 4.0 to 7.0 Gm. daily, trying to maintain a blood level in excess of 2.5 mg. per cent. We can recommend this combination of topical and internal therapy for certain severe pyodermas that do not respond to the usual older measures.

In agreement with observers in other fields of medicine, we have found that, in general, it may be stated that the more acute the dermatosis, the better the results with sulfonamide therapy. Either *sulfanilamide* or *sulfapyridine* has been reported to be definitely useful in the following dermatologic conditions, among others: (1) streptococcic cellulitis and lymphangitis; (2) streptococcic ulcers; (3) erysipelas; (4) erythema multiforme (some types); (5) chancreoid; (6) lymphogranuloma venereum; (7) subacute discoid type of lupus erythematosus. There have been many interesting reports of further uses of sulfonamides in isolated cases or in too short a series to be conclusive evidence for general acceptance. Anderson³¹ and Abramowitz³² report cures of patients with acute disseminated lupus erythematosus. Glyn-Hughes and Spence³³ report a series of 12 patients with lupus erythematosus in whom all gave favorable responses and six were cured with sulfapyridine. On the other hand, Wile and Holman³⁴ report discouraging results with sulfonamides in acute disseminated lupus erythematosus.

Dobson³⁵ believes sulfanilamide a valuable adjunct in the treatment of actinomyces. Sehoeh and Shelmire³⁶ report success with sulfanilamide in the treatment of erysipeloid of Rosenbach.

Good initial results in dermatitis herpetiformis and sycosis vulgaris have seldom been maintained.

It is almost an impertinence here to discuss the general systemic signs and symptoms of the toxicity of the sulfonamides, since they have been so widely described and so accurately detailed. The cutaneous reactions usually seen are the well-known morbilliform eruptions and the less known dermatitis from photosensitivity, *erythema multiforme-like* and *erythema nodosum-like* dermatoses, scarlatiniform eruptions, fixed eruptions, stomatitis, urticaria, purpura, varioliform eruptions, and exfoliative dermatitis.³⁷ Sulfathiazole in many instances presents a conjunctivitis or "pink eye" which may be the initial—or even the only—manifestation of intolerance. Baer states he has observed generalization in two patients with psoriasis following a sulfonamide eruption.³⁸ He believes this to be due to a Koebner phenomenon at the site of local injury due to the eruption. This observation is well to keep in mind with reference to other photosensitizing agents that may be ingested by psoriatics undergoing ultraviolet light treatments.

HORMONAL THERAPY (ENDOCRINOLOGIC THERAPY)

The present enthusiasm for endocrine therapy is on a par with that for the sulfonamides and vitamins. Many liters of *estrogenic substances* and of *anterior-pituitary-like substances* are being administered to women presenting various dermatoses at the menopause. However, specific treatment with *estrogens* in dermatology is almost limited to *senile pruritus* and *kraurosis in the female*. As early as 1935 Davis³⁹ reported good results with suppositories of 75 R.U. of amniotin (Squibb) in kraurosis. Recently stilbesterol has been reported to be highly effective when given by mouth in doses ranging from 0.1 to 10 mg. daily; or, topically, in ointments containing 10 to 20 mg. per ounce.⁴⁰ (In the way of non-endocrinologic treatment Saville⁴¹ claims excellent results in both simple leucoplakia and kraurosis with diathermy, using a belt about the hips or a pubic plate and an all-metal electrode high in the vagina. Relief is obtained in four to fifteen treatments, using the factors fifteen minutes and 0.7 to 1.5 amperes.) Lane⁴² reports the clearing of an *oral leucoplakia* in two patients by use of estradiol.

Folliculin has been advised in *alopecia* for both men and women. *Testosterone* is known to grow hair, sometimes on face and chest, to the embarrassment of young women. We have recently seen several cases of young women treated with testosterone for menorrhagia who presented new growth of hair on face and body, deepening of voice, and a deep pustular acneform eruption, most marked on borders of the face. Moreover, in patients with a tendency to acne, or even in those with only mild seborrheic habitus, many of the androgenic hormones will quite regularly produce acne. The same remark applies to eunuchs or eunuchoid persons receiving androgens.⁴³ Many of the androgen acnes we have observed have cleared up promptly upon withdrawal of the hormonal therapy (in a few days to weeks). However, a few cases have persisted for many months despite the stopping of the hormonal administration and the usual anti-acne therapy.

Feldman⁴⁴ states that six patients with generalized senile pruritus were cured by injections of 5 to 15 mg. testosterone propionate weekly. There are as yet no reports by others to confirm this interesting and important observation.

Brittleness and friability of nails can occasionally be benefited by *thyroid* therapy. Subclinical thyroid hypoaetivity may be manifested by no other clinical signs than mild dryness of the skin and hair, or brittleness of the nails. In our experience a fair number of patients with *acne vulgaris* or with *atopic dermatitis* will respond favorably to thyroid medication even though the basal metabolic rate is within the so-called normal limits.

ANTISYPHILITIC THERAPY

In our opinion the outstanding advance in antisyphilitic treatment is the massive arsenotherapy in early syphilis by some of the newer methods, including the continuous intravenous drip method.⁴⁵ The idea of Sterilisatio Magna began with Ehrlich, and was carried on in the experiments of Linser⁴⁶ and Pollitzer.⁴⁷ A modern study, begun by Chargin, Leifer, and Hyman in 1932,⁴⁸ is a masterpiece of painstaking basic preliminary work and careful clinical and laboratory observation during the treatment. The conservative conclusions

expressed by the whole group at this time are characteristic of the truly scientific attitude of the workers. The original article⁴⁵ is on the "must list" for anyone even remotely interested in syphilis or serology. Much of the data concerning serologic comparisons and interpretations has not as yet been published, and will, undoubtedly, of itself be an important contribution. It is impossible to summarize this work since the original report is, indeed, a summary. Certain conclusions regarding the possible advantages of the massive intracutaneous drip method may, however, be safely stated: (1) More patients who began treatment are cured than with the intermittent treatment (during which so many patients disappear as soon as rendered asymptomatic, only to develop later the well-known serious aftermaths of insufficient therapy). (2) The method, when mapharsen is employed, is as safe, or *almost* as safe, as the intermittent treatment in early syphilis. (3) Nitritoid reactions have been almost proved to be a "speed shock" phenomenon and are not seen in this procedure. (4) Mapharsen, as previously believed, has proved efficacious and less toxic than neoarsphenamine. (5) No major dermatitides were seen with mapharsen. (6) Serious manifestations of toxicity occur with neoarsphenamine—hemorrhagic encephalitis, peripheral neuritis, hepatitis. (7) Relapses do occur. (8) The late results, including both those due to the syphilis itself and to massive dose arsenic therapy, are not yet known. (9) It is, as yet, only an experiment, but nevertheless, an intensely interesting and valuable one.

However, as one of us (M. B. S.) has previously stated: "It is, for example, not yet known whether mapharsen could not be given several times daily by means of small syringes with effects equivalent to those obtained when the same dose is given by continuous drip."⁴⁶ At present it appears to us probable that the results obtained with mapharsen in the intravenous drip may be due not so much to the beneficial effects of the *slow drip method*, as to a *previously unrecognized lack of toxicity or broad therapeutic index of mapharsen itself in man*. If this theory proves true, one should be able to give daily large doses of mapharsen in ambulatory patients, with small syringes and without the difficulties and expense of hospitalization and drip administration.

Very recent studies, particularly those of Sehoeh and Alexander,⁵⁰ and collaborators, support the statement just quoted from the Year Book and our present opinion. For these investigators have now treated over 40 patients with early syphilis with *daily doses of 0.12 Gm. of mapharsen*. Each dose was given rapidly in 10 c.c. of water with a small syringe. The patients all were ambulatory, all continued with their occupations; none experienced the slightest untoward effects or untoward reactions. In this as yet small series the results are most promising. The disappearance of the syphilitic manifestations and the reversal of the serologic reactions were fully as good as with the drip method.

A further advance in antisyphilitic therapy is the introduction of the drug *sobisminol*,⁵¹ which can be given orally (as well as by injection) and orally compares favorably in its action with injectable bismuth preparations. However, the bismuth in this form of medication is eliminated rapidly. No depot or constant source of absorption is established with sobisminol,⁵² and *clinical relapse* has been reported on its substitution for injectable bismuth. In one

report it has seemed to have an especially beneficial influence in patients with late neurosyphilis of the tabetic type.⁵³ This benefit apparently regresses with cessation of treatment, but the drug can be given for months without signs of cumulative effects. This does not mean that it is without either major or minor toxic effects of bismuth. In our hands it has been used with gratifying results in two patients with lupus erythematosus, and in one patient with lichen planus. Other authors have reported similar good results.

The discovery by Cole, De Ore, Driver, Johnson, and Schwartz⁵⁴ represents not only a great therapeutic advance but a singularly fascinating subject for further theoretical and practical studies. These observers found that certain bismuth salts, particularly *thiobismol*, control the bouts of therapeutic malaria in the most effective manner. By controlled administration the patient can be kept free of fever and paroxysms whenever desired, and by stopping the drug the paroxysms can be allowed to recur whenever indicated. In other words, the bismuth salt keeps the patient free of malarial attacks but does not destroy the infesting plasmodia, or at least not to such a degree as to eliminate the disease; so that, after the desired interruption, the malarial treatment can be resumed without the necessity of a reinoculation. The theoretic interest as well as the practical advantages of this discovery are both great and obvious.

Saenz, Triana, and Armenteros⁵⁵ have discovered in *pinta* patients a *spirochete* morphologically identical to the *S. pallida* and *S. pertenuis*. This disease has long been known to possess most of the serologic characteristics of syphilis and the discovery reawakens or intensifies the interest in the study of cross immunity between syphilis, frambesia, and *pinta*. It is the ambition of every dermatosyphilologist to find an innocuous disease which will give a cross immunity to syphilis, paralleling that achieved in smallpox with cowpox virus (vaccine). However, little is as yet known about the immunologic relationships or the effects of extracts of microorganisms and of attenuation procedures in various human spirochetoses.

A promising aid in antisyphilitic therapy is embodied in the report by MacKee and Astrachan⁵⁶ of the mitigating influence of whole crude liver extract on the erythrodermas incident to antisyphilitic treatment. It was even found that certain individuals could continue receiving cautiously administered arsenical treatment after a major arsenical dermatitis if crude liver extract were given intramuscularly. This report represents a confirmation of, and an addition to, the findings of Genner⁵⁷ and others.

We believe the combination of a titered Kolmer, Wassermann, and Kline diagnostic and exclusion tests are sufficient, in almost every instance, for serologic diagnostic purposes and for evaluation of treatment results. The Laughlen test, with the three-minute serum inactivation modification,⁵⁸ may be valuable for pretransfusion tests.*

MISCELLANEOUS THERAPY AND PROPHYLAXIS

The recent work on *poison ivy* dermatitis assumes tremendous added importance when considered in connection with the defense program. A majority of

*The use of antigen prepared from spirochetes, as developed by Gaechtgen and reported by Erickson and Eagle (Abstract, Year Book of Dermat. and Syph., p. 733, 1941) may prove a decided advance in diagnostic serologic tests for syphilis.

the military trainees are likely to be "ivy sensitive," and to be exposed this summer. Shelmire⁵⁹ has found that the dermatitis-producing excitant is not an oil, but a dialyzable fraction of the oleoresin, soluble in water and in urine. He also reports that the excitant is not carried in smoke, as previously believed. A large and comprehensive study of *oral desensitization* is now being carried out on a nationwide scale, due to Shelmire's experiments in oral prophylaxis, which represent an extension and modification of the older work of Jay Schamberg, Strickler, and others.

Schwartz and Warren⁶⁰ have developed and advocated the use of a *sodium perborate-containing cream* for the prevention of poison ivy dermatitis. While these authors submit observations supporting the efficacy of the cream, Shelmire, on the other hand, presents experiments tending to show its lack of effective protection.⁶¹ The value of this cream is, therefore, still awaiting the final judgment of practical results on large series of exposed persons.

The treatment of *chronic ulcers* with *zinc peroxide paste* or *mass* is a valuable and relatively recent therapeutic measure. Rigid attention to selection of the powder—since not all zinc peroxide is active—preparation of the "mass" and of its application must all be observed. An excellent presentation of the rationale and technique is found in the paper of Meleney and Harvey.⁶²

We have found application of the *Aloe vera* leaf⁶³ *alva gel*,⁶⁴ and cod allantoin ointment effective in some chronic ulcers, especially the use of the leaf in some of the ulcers following radiodermatitis.

Pliofilm mitts and sheets are useful in retaining wet dressings of all types.⁶⁵

The development of a refined test material (*Lygranum*) for the diagnosis of *lymphogranuloma venereum* (*lymphopathica venerea*) by the method of Rake, McKee, and Shaffer,⁶⁶ promises to be a notable advance. In this procedure the yolk sac of the chick embryo is inoculated, and a practically pure and highly concentrated test material is obtained. We suggest that the Frei test, with original human material, and tests with this new material, be performed in parallel and in a comparative manner in a large series of cases of (1) acute and chronic, suppurative and nonsuppurative inguinal adenitis; (2) acute and chronic proctitis; (3) fistulas of rectum and genitals; (4) acute and chronic ulceration of the genitals; (5) localized elephantiasis.

Semon and Hermann⁶⁷ report improvement in *acne vulgaris* in both men and women following the administration of either protamine zinc or ordinary *insulin*. Dosage was usually 5 to 15 U. three times weekly, given without any adjuvant treatment. Exacerbations occurred upon cessation of treatment and improvement began within forty-eight hours of its resumption. The insulin therapy sprang from observations of the lag in sugar tolerance curves in acne patients. The authors state it is impossible, at the present time, to "differentiate between metabolic vagaries and disturbance of hormonal balance."

A recent innovation in the treatment of *intractable pruritus* is the *tattooing* of the anal area with red sulfide of mercury (Hollander, E.^{68a} and Turrell, R.^{68b}). Good results are reported in the majority of cases.

The use of trichophytin in erysipelas-like infections associated with dermatophytosis of the feet, as advocated by Sulzberger, Rostenberg, Jr., and

Goetze,⁶⁹ is worth trying. Individuals in whom a favorable response is expected are those showing an *immediate wheal reaction* to intracutaneous test. The dose is 0.1 c.c. of the usual 1:30 dilution given intracutaneously at five- to seven-day intervals.

The work of Becker⁷⁰ and others with *lipocaic* (Dragstet and co-workers), although apparently productive of no lasting results in *psoriasis*, is extremely interesting. The substance is a neutral, insulin-free and fat-free alcoholic extract of pancreas which apparently has selective power of converting some fats to glucose.

Conrad, Conrad, Mapother, and Weiss⁷¹ report the use of bismarsen as a valuable addition to the therapy of *lichen planus*; 0.1 Gm. given intramuscularly twice weekly exerted a favorable influence on the ordinarily refractory lesions of the mouth, of the penis, and of the hypertrophic type. Vitamin C should be administered while the bismarsen is being given.

In closing, it may be appropriate to give some direct advice from Becker:⁷² "Therapy consists, first of all, in removing precipitating factors, social and environmental; secondly, in relieving the underlying instability and exhaustion; and thirdly, in application of orthodox measures."

The principles enumerated are not only good but are long established. However, there is much room for discussion regarding the order in which Becker here mentions these principles. If the order of mention is to be that of relative importance, this will depend not only upon the training, viewpoint, and inclination of the physician, but also upon the particular constellation of circumstances bearing upon the particular case at the particular moment.

REFERENCES

1. Sulzberger, M. B.: Discussion, Symposium on Vitamins, Bronx Der. Soc., December 26, 1940, Arch. Dermat. & Syph. In Press.
2. Editorial Comment, Goodman, J., Kanof, N. M., Wise, F., and Sulzberger, M. B.: Year Book of Dermat. & Syph., Chicago, 1938, Year Book Publishers, p. 467.
3. Peck, S. M., Chargin, Louis, and Sobotka, Harry: Keratosis Follicularis, Arch. Dermat. & Syph. 43: 223, 1941.
4. Carr, F. H., and Price, E. A.: Colour Reactions Attributed to Vitamin A, Biochem. J. 20: 497, 1926.
5. Herkt, S., and Mandelbaum, J.: Dark Adaptation and Experimental Human Vitamin A Deficiency, Am. J. Physiol. 130: 651, 1940.
6. Brunsting, L. A., and Sheard, Charles: Dark Adaptation in Pityriasis Rubra Pilaris, Arch. Dermat. & Syph. 43: 42, 1941.
7. Patek, A. J., Jr., and Haig, C.: The Occurrence of Abnormal Dark Adaptation and Its Relation to Vitamin A Metabolism in Patients With Cirrhosis of Liver, J. Clin. Investigation 18: 609, 1939.
8. Andrews, George C.: Pennsylvania M. J. 43: 1535, 1940.
9. Gross, Paul: Arch. Dermat. & Syph. In Press.
10. Madden, J. F.: The Treatment of Psoriasis, J. A. M. A. 115: 588, 1940.
11. Forsyth, J.: Pink Disease Treated by Vitamin B, M. J. Australia 2: 751, 1939.
12. Sydenstricker, V. P., Sebrell, W. H., Cleckley, H. M., and Kruse, H. D.: Ocular Manifestations of Ariboflavinosis, J. A. M. A. 114: 2437, 1940.
13. Smith, Susan G., Smith, David T., and Callaway, J. L.: Dysfunction of the Sebaceous Glands in Pellagra, J. Invest. Dermat. 4: 23, 1941.
14. King, J. D.: Vincent's Disease Treated With Nicotinic Acid, Lancet 2: 32, 1940.
15. György, Paul: Dietary Treatment of Scaly Desquamative Dermatoses of the Seborrheic Type, Arch. Dermat. & Syph. 43: 230, 1941.
16. Spies, T., and others: Pantothenic Acid in Human Nutrition, J. A. M. A. 115: 523, 1940.
17. Kristensen, K. P., and Vendel, S. N.: Treatment of Eczema With Vitamin B Complex, Lancet 1: 170, 1940.
18. Andrews, George C.: Opus cited—v. 8.

18. Gross, Paul: Discussion, Symposium on Vitamins, Bronx Der. Soc., December 26, 1940, Arch. Dermat. & Syph. In Press.
19. Sjögren, H.: Cited by Stahel, Klin. Wehnschr. 17: 1692, 1938; Sutton, R. L., and Sutton, R. L., Jr., Diseases of the Skin, St. Louis, 1939, C. V. Mosby Co., p. 462.
20. Saunders, T. S.: Vitamin Therapy in Dermatologic Practice, Northwest Med. 39: 209, 1940.
21. Scarborough, H.: Deficiencies of Vitamin C and Vitamin P in Man, Lancet 2: 644, 1940.
22. Gorrie, D. R.: Purpura Hemorrhagica After Arsenic Therapy Treated With Vitamin P, Lancet 1: 1005, 1940.
23. Hiramatsu, Noluio: Treatment of Purpura With Vitamin P, Jap. J. Dermat. & Urol. 46: 105, 1939.
24. Wise, F., and Sulzberger, M. B.: Editorial Comment, Year Book of Dermat. & Syph., Chicago, 1940, Year Book Publishers, p. 483.
25. Wechsler, I. S.: Treatment of Amyotrophic Lateral Sclerosis With Vitamin E, Am. J. M. Sc. 200: 765, 1940.
26. Baer, R. L.: Case Presentation, Bronx Der. Soc., Feb. 27, 1941.
György, Paul: Opus cited—v. s. 13.
27. Silver, Henry, and Chiego, Bernard: Nails and Nail Changes, J. Invest. Dermat. 3: 369, 1940.
28. Gross, Paul: The Role of the Unsaturated Fatty Acids in the Aerodynia (Vitamin B, Deficiency) of the Albino Rat, J. Invest. Dermat. 3: 505, 1940.
29. Mellon, R. R., Locke, A. P., and Shinn, L. E.: Anti-enzymatic Nature of Sulphanilamide's Bacteriostatic Action, Am. J. M. Sc. 199: 749, 1940.
30. Mellon, R. R.: Clinical and Experimental Aspects of Mode of Action of Sulfanilamide and Sulfapyridine Compounds, Ohio State M. J. 36: 1265, 1940.
31. Anderson, H. T.: Fulminating Acute Lupus Erythematosus Cured by Sulfanilamide, Arch. Dermat. & Syph. 38: 621, 1939.
32. Abramowitz, E. W.: Discussion on Wisc, Fred: Discoid Lupus Erythematosus Undergoing Dissemination, Arch. Dermat. & Syph. 38: 661, 1938.
33. Glyn-Hughes, F., and Spence, A. M.: Sulfonamide Therapy in Lupus Erythematosus, Brit. M. J. 2: 741, 1940.
34. Wile, Udo, J., and Holman, H. H.: Acute Disseminated Lupus Erythematosus, Arch. Dermat. & Syph. 42: 1059, 1940.
35. Dobson, I., Holman, E., and Cutting M.: Sulfanilamide in Therapy of Actinomyces, J. A. M. A. 116: 272, 1941.
36. Schoch, Arthur G., and Shelmire, Bedford: Erysipeloid of Rosenbach Successfully Treated With Sulfanilamide, Arch. Dermat. & Syph. 41: 570, 1940.
37. Hughes, Raymond P.: Sulfanilamide in Dermatology, Arch. Dermat. & Syph. 42: 39, 1940.
38. Baer, R. L.: Personal communication.
39. Davis, M. E.: Treatment of Senile Vaginitis With Ovarian Follicular Hormone, Surg., Gynec. & Obst. 51: 680, 1935.
40. Abarbanel, A. R., and Klein, M. O.: Clinical Experiences With Stilbestrol, New York State J. Med. 41: 383, 1941.
41. Saville, Agnes: Treatment of Pruritus Vulvae, Leukoplakia and Kraurosis, Brit. J. Dermat. & Syph. 52: 353, 1940.
42. Lane, C. Guy: Discussion of Case of G. M. Crawford, Arch. Dermat. & Syph. 43: 182, 1941.
43. Hamilton, James B.: Male Hormone Substances, Inductors of Acne. Read at Third Annual Meeting, Society for Investigative Dermatology, June 11, 1940.
44. Feldman, Samuel: Discussion of Case, Bronx Der. Soc., Feb. 27, 1941.
45. Baehr, George, Leifer, W., Chargin, Louis, Hyman, H. T., Mahoney, J. F., Webster, Bruce; Thomas, Evan; Sobotka, Harry; Mann, Walter; Feldbou, Edith; and Rice, John L.: Arch. Dermat. & Syph. 42: 239, 1940.
46. Linser, Paul: Handb. d. Haut-u. Geschlechtskr., Berlin, Julius Springer, Vol. 18, p. 593, 1940.
47. Pollitzer, S.: The Principles of Treatment of Syphilis, J. Cutan. Dis. 24: 633, 1916.
48. Chargin, Louis, Leifer, W., and Hyman, H. T.: Studies on Velocity and Response to Intravenous Injections, etc., J. A. M. A. 104: 878, 1935.
49. Wise, Fred, and Sulzberger, M. B.: Year Book of Dermat. & Syph. Editorial Comment, Chicago, 1940, Yearbook Publishers, p. 640.
50. Schoch, Arthur and Alexander, Lee J.: Personal communication. See also article in press, Am. J. Syph., Gonorr., & Ven. Dis., 1941.
51. Report of Council of Pharmacy and Chemistry, J. A. M. A. 113: 2235, 1939.
52. Hanzlik, P. J., Lehman, A. J., Richardson, A. P., and Van Winkle, W., Jr.: Clinical Ecretion of Bismuth After Oral Administration of Sobilminol, Arch. Dermat. & Syph. 36: 708, 1937.

53. Scholtz, Julius R., McEachern, Katherine D., and Wood, Clyde: Sobisminol Mass: Clinical Results With Oral Administration, *J. A. M. A.* 113: 2219, 1939.
54. Cole, H. N., De Oreo, G. A., Driver, J. R., Johnson, H. H. and Schwartz, W. F.: Use of Bismuth Injections to Manage Course of Therapeutic Malaria, *J. A. M. A.* 115: 422, 1940. Year Book of Dermat. & Syph., Chicago, 1940, Yearbook Publishers, p. 659.
55. Saenz, B., Triana, J. G., and Armenteros, J. A.: Pinta in Cuba, *Arch. Dermat. & Syph.* 41: 463, 1940.
56. MacKee, G. M., and Astrachan, G. D.: The Value of Liver Extract in Cases Intolerant to Arsenicals, Heavy Metals, and Radiation, *J. Invest. Dermat.* 3: 5, 1940.
57. Genner, Vigo: By-Effects in Salvarsan Therapy and Their Prevention, Copenhagen, 1936, Levin & Munksgaard.
58. Stecker, John F., Greely, P. H., and Paul, E. J.: Laughlen Test for Syphilis, *Arch. Dermat. & Syph.* 43: 317, 1941.
59. Shelmire, Bedford: Poison Ivy Dermatitis, *Arch. Dermat. & Syph.* 42: 399, 1940.
60. Schwartz, Louis, and Warren, L. H.: Protective Ointments for Prevention of Poison Ivy Dermatitis, *Public Health Rep.* 55: 1327, 1940.
61. Shelmire, Bedford: Sodium Perborate Ointment and Poison Ivy Dermatitis, *J. A. M. A.* 116: 681, 1941.
62. Meleney, Frank L., and Harvey, Harold D.: Combined Use of Zinc Peroxide and Sulfanilamide in Treatment of Chronic, Undermining, Burrowing Ulcers Due to Micro-Aerophilic Hemolytic Streptococcus, *Ann. Surg.* 110: 1067, 1939.
63. General and Marine Laboratories, Crisfield, Md.
64. *Ibid.*
65. Stryker, Garold V., and Grindon, Joseph, Jr.: Convenient Method of Applying Wet Dressings, *J. A. M. A.* 115: 121, 1940.
66. Rake, G., McKee, C. M., and Shaffer, M. F.: *Proc. Soc. Exper. Biol. & Med.* 43: 332, 1940; 44: 410, 1940.
67. Semon, Henry C., and Hermann, Franz: Insulin Therapy in Aene Vulgaris, *Brit. M. J.* 52: 123, 1940.
68. (a) Hollander, E.: Treatment of Pruritus Ani by Tattoo With Mercuric Sulfide, *Arch. Dermat. & Syph.* 38: 337, 1938.
 (b) Turell, Robert: Tattooing (Puncturation) With Mercury Sulfide and Other Chemicals for the Treatment of Pruritus Ani and Perineal, *J. Invest. Dermat.* 3: 389, 1940.
69. Sulzberger, M. B., Rostenberg, Adolph, Jr., and Goetze, Dorothy: Recurrent Erysipelas-Like Manifestations of the Legs, *J. A. M. A.* 108: 2189, 1937.
70. Becker, S. W., Walsh, Edmund N., Clarke, D. E., and Dragstedt, L. R.: Further Studies on the Use of Lipoenic in the Treatment of Psoriasis, *J. Invest. Dermat.* 4: 59, 1941.
71. Conrad, Conrad, Jr., Mapother, P., and Weiss, Richard S.: Liehen Planus Treated With Bismuth Arsphenamine Sulfonate (Bismarsen), *South. M. J.* 33: 721, 1940. Abstract in the Year Book of Dermat. & Syph., p. 496, 1940.
72. Becker, S. W.: Editorial, Digest of Treatment 3: 521, 1940.

CLINICAL AND EXPERIMENTAL

THE EFFECT OF THE SUBCUTANEOUS ADMINISTRATION OF PROTAMINE (SALMINE) TO RABBITS AND MICE*

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FOLLOWING the announcement of Hagedorn¹ in 1935 that the addition of protamine greatly prolongs the action of insulin, this substance has been used widely as a constituent of protamine insulin and protamine zinc insulin. If used in small doses, protamine apparently has no deleterious effect upon the organism. However, since over a period of years many diabetic patients take relatively large quantities of insulin, the question has arisen as to how large amounts of protamine insulin can be given safely.

Forty years ago Thompson,² working in Kossel's³ laboratory, reported the results obtained when protamine was injected intravenously into ureotized dogs. He found that a dose of elupeine (originally regarded as chemically identical with salmine) greater than 15 to 18 mg. per kilogram could not be given without causing death of the animal. Following the injection of protamine a definite and relatively prompt fall of the blood pressure took place. After a nonfatal dose, the blood pressure regained the normal level in twenty-five to thirty minutes. Simultaneous with the fall in the blood pressure after a nonfatal dose, the respirations at first became increased in rate and depth; then followed a period of rest and finally gradual resumption of normal breathing. It was noteworthy, however, that active thoracic breathing was absent as long as the effect of the protamine lasted. In addition to the effects upon the blood pressure and the respiration, Thompson noted delayed clotting of the blood and a diminution in the number of circulating leucocytes.

Jappelli⁴ in 1933 repeated Thompson's experiments in unanesthetized animals. Doses of salmine of 20 mg. per kilogram of animal caused agitation, dyspnea, salivation, diarrhea, and a drop in the arterial blood pressure. A second dose caused a further drop in arterial pressure, and a third was fatal (vasomotor paralysis).

According to Walther and Ammon,⁵ three guinea pigs of 250 to 300 Gm. body weight survived two intraperitoneal injections each of 5 to 10 mg. of protamine. They died, however, following intraeardial injections of 35 mg. four weeks later.

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Dr. Vartiainen was the holder of a fellowship of the Finnish Government.

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Because in the literature there are no data regarding the toxicity of protamine when given subcutaneously, we have determined the lethal dose for mice and rabbits and have studied the signs and symptoms which follow the administration of large amounts.

RESULTS

Lethal Doses.—The lethal dose following subcutaneous injection was determined in 40 mice and 23 rabbits.

Female mice weighing from 12.0 to 33.5 Gm. were used. Food and water were withheld during the first twelve hours after the administration of protamine, but were allowed before and after that time. The neutralized protamine* was given subcutaneously in 1 per cent concentration in a physiologic solution of sodium chloride. The behavior of the animals was observed for five days after the injections.

As may be seen in Table I, the average or median lethal dose of protamine in our experiments was about 250 mg. per kilogram of animal. All deaths occurred within one hour after injection.

TABLE I

MORTALITY OF MICE AFTER A SINGLE SUBCUTANEOUS INJECTION OF PROTAMINE (SALMINE)

DOSE PER KG. ANIMAL (MG.)	NUMBER OF MICE TESTED	NUMBER OF MICE DYING	TIME OF DEATH AFTER INJECTION (MIN.)
400	2	2	18-32
300	10	8	19-55
250	10	5	23-60
200	10	1	40
100	4	0	
50	2	0	
25	2	0	

TABLE II

MORTALITY OF RABBITS AFTER A SINGLE SUBCUTANEOUS INJECTION OF PROTAMINE (SALMINE)

DOSE PER KG. ANIMAL (MG.)	NUMBER OF RABBITS TESTED	NUMBER OF RABBITS DYING	TIME OF DEATH AFTER INJECTION (HR.)
500	2	2	4-10
300	8	3	8-16
200	5	3	5-12
100	4	0	
50	2	0	
25	2	0	

Male rabbits weighing 1,180 to 2,760 Gm. and fasted twenty-four hours were used. No food or water was allowed during the first twelve hours after injection of protamine. The behavior of the animals was observed during the following five days. The protamine was injected in 10 per cent concentration in a physiologic solution of sodium chloride. Before injection these preparations were warmed to about body temperature in order to diminish the turbidity which was present in the cold.

*The protamine used was salmine (Lot No. 961271) prepared by Eli Lilly & Co., Indianapolis, and furnished to us without cost.

The median lethal dose of salmine injected subcutaneously seemed to be essentially the same in rabbits as in mice (Table II), being between 200 and 300 mg. per kilogram of animal (a dose of 250 mg. per kilogram was not used with the rabbits). However, the results were not as uniform as in the mouse experiments; it is possible that this may have depended upon the more delayed and uneven absorption of protamine when injected in 10 per cent, rather than 1 per cent, concentration. In contrast with the experience with mice, only one of the rabbits died during the first four hours after an injection.

Signs and Symptoms Following the Subcutaneous Injection of Large Amounts of Protamine.—One of the most striking early signs noted following the subcutaneous injection of salmine was the dilatation of superficial blood vessels, as seen particularly in the ears of rabbits. This was first observed usually fifteen to twenty minutes after an injection and reached its maximal intensity thirty to ninety minutes after the injection. Although barely demonstrable after the smaller doses used (50 to 100 mg. per kilogram), it was marked after the larger doses. The large ears of white New Zealand rabbits became warm and strikingly bluish red.

Beginning with the third hour after injection, there appeared in some animals certain signs of blood vessel damage, especially after doses larger than 200 mg. per kilogram. Thus pressure upon the ear with the finger tips produced blanching which persisted for an unusually long time. The prominence of this feature depended somewhat upon the degree of dilatation of the superficial blood vessels at the time pressure was applied. In a few cases there was to be seen at about the fourth hour following injection a marked edema of the ears which remained visible the following day.

In most of the fatal cases the rabbits had marked respiratory distress, beginning one to two hours after the injection. Breathing became very labored and slower than normal. Sometimes râles could be heard, suggesting pulmonary edema. At this stage the animals often assumed a position of opisthotonos, with the nose lifted up and the ears directed along the back. At times this position was maintained by supporting the chin on a base, as on the edge of a basket or pan. After doses of 100 to 500 mg. per kilogram there sometimes occurred rigidity of the animal, which reached a maximum in the fifth to seventh hour after injection, and occasionally could still be seen the following day.

Following doses of from 50 mg. per kilogram and more, the animals seemed to suffer from marked malaise between the first to fifth hours and after large doses this persisted even longer.

Between the second and sixth hours many rabbits showed a definite increase in the output of urine; this was usually very light and clear. The urine occasionally reduced Benedict's solution but to no greater extent than did 0.15 per cent glucose. During a period in which rabbit P16 (dose of protamine, 200 mg. per kilogram) had marked hyperglycemia, no urine was obtained for sugar determinations. Rabbits P22 and P25 (dose of protamine, 300 and 200 mg. per kilogram, respectively), showing marked changes in the kidneys at post mortem, had in the urine albumin, granular casts, red blood corpuscles, white blood cells, and epithelial cells.

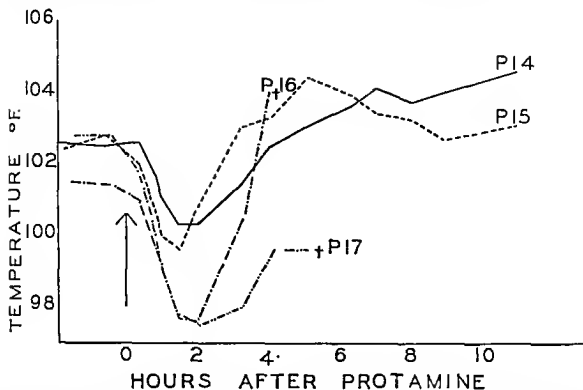


Chart 1.—Effect of the subcutaneous injection of protamine on the rectal temperature of rabbits. Animals P14 and P15 received 100 mg., and P16 and P17 200 mg., per kilogram of body weight. The sign + indicates death of the animal.

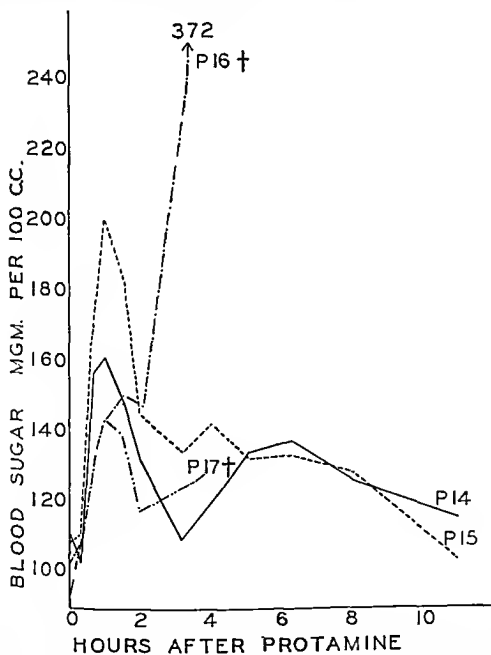


Chart 2.—Effect of the subcutaneous injection of protamine on the blood sugar of rabbits. Animals P14 and P15 received 100 mg., and P16 and P17 200 mg., per kilogram of body weight. The sign + indicates death of the animal.

One of the rabbits (P25) developed, after the fifth hour following injection, paralysis of left anterior and right posterior limbs.

Some of the animals showed between the first and third hour the frequent defeecation of a moist mucous fecal mass.

In a group of eight rabbits the changes in body temperature after the injection of protamine were studied by hourly rectal measurements. The largest doses used in these experiments, 200 mg. per kilogram, caused a marked fall of body temperature within two hours after the injection of protamine. After that, the temperature rose again, reaching levels above normal. Even the smaller doses (25 to 100 mg. per kilogram) had a similar effect, that of 25 mg. per kilogram being very slight.

The blood sugar, studied in the same eight rabbits, showed marked alterations (samples of 0.1 c.c. were taken from a marginal ear vein, and the sugar content was determined according to the method of Folin and Malmros⁶). During the first two hours the values were increased, showing a maximum usually at one hour after the injection of protamine. During a second rise, which began usually in the third hour, one of the animals (P16) had premortal values as high as 372 mg. per 100 c.c.

The symptoms in mice after injections of protamine corresponded to those in rabbits, although they were more difficult to follow accurately. After a dose of 200 to 400 mg. per kilogram of animal, all the surviving mice seemed very ill during the first one to five hours. Six animals were opisthotonic about ten to fifty minutes after the injection. Many mice had marked rigidity of the body about one hour after the injection. Some of the animals had clonic convulsions a short time before death; others died with the body flaccid.

After a dose of 50 to 100 mg. per kilogram of animal, the mice seemed to suffer malaise for a few hours. The smallest dose of 25 mg. per kilogram seemed to have no effect upon the animals.

*Post-mortem Findings.**—The most striking and constant finding on examination of the rabbits which died were hemorrhages in the thymus gland. These varied in extent from areas of pin-point size to massive involvement of the gland. The extent of the hemorrhages did not seem to depend absolutely upon the size of the dose of protamine used, although all animals dying had received relatively large amounts. The lungs showed many pleural and parenchymatous hemorrhages of varying size, and frequently there was bloody foam in the trachea and bronchi. The changes in the lungs seemed to parallel roughly in extent those in the thymus. The kidneys showed cortical and subcortical foci of hemorrhage and necrosis, suggesting infarcts, from pin-point size to 5 mm. In mice the most constant findings were in the lungs, which were edematous and often showed tiny points of hemorrhage on the surface. Hemorrhages were noted also on the surface of the thymus and adrenal glands.

Microscopic study of the tissues confirmed the findings made on gross examination. In addition, the liver showed some degree of focal necrosis with polymorphonuclear infiltration; in one instance the process was widespread with

*The necropsies reported in this paper were carried out immediately after the spontaneous death of the animals. Tissues were placed in formalin or Zenker's fluid.

involvement of about half of the liver tissue in the sections examined. Degeneration of the tubular epithelium of the kidney with many hyaline casts in the tubules was seen. In one rabbit a small area of focal necrosis with polymorphonuclear infiltration was seen in one adrenal gland.

DISCUSSION

The data presented in this paper demonstrate that protamine (salmine) is definitely toxic when given subcutaneously in large amounts. However, the doses required for the appearance of abnormal signs are relatively enormous.

A dose of protamine zinc insulin of 100 units is from two to three times as great as that used daily by a patient with diabetes of moderate severity. According to present standards, 1.25 mg. of protamine are contained in each 100 units of protamine zinc insulin. For a patient weighing 65 kg. this would represent roughly 0.02 mg. per kilogram, or about $\frac{1}{10,000}$ the minimal lethal dose as established in our work for mice and rabbits. Furthermore, the tendency to toxic effect probably is lessened because of the tolerance gradually set up by the daily subcutaneous administration. At any rate, in an extensive use of protamine-containing insulin in hundreds of cases of diabetes treated since 1935 at the George F. Baker Clinic, no harmful effects have been observed which could be attributed to the protamine per se. Local responses consisting of redness, swelling, heat, soreness, and itching at the site of injection are frequently seen, but even these effects usually become less and disappear after injections have been continued for several days or at the most, a few weeks.

Although we have regarded these local responses to protamine zinc insulin as allergic in nature, Kern and Langner⁷ doubt this because they obtained negative intracutaneous reactions with a protamine solution in 104 diabetic patients treated with protamine zinc insulin. They quote Taylor⁸ and Wells⁹ to the effect that protamines have no antigenic properties; furthermore, they were unable to induce sensitivity to protamine in four guinea pigs.

In an article already mentioned, Walther and Ammon⁵ interpret the death of three guinea pigs, each of which was given two parenteral injections of protamine four weeks apart, as due to anaphylactic shock. It is possible that the fatalities observed by Walther and Ammon occurred on a purely toxic, rather than an allergic, basis. Walther and Ammon gave as their second or "shocking" dose, 35 mg. of protamine to a guinea pig weighing 250 to 300 mg., or approximately 115 to 140 mg. per kilogram. Although this is somewhat below the median lethal dose which we found in mice and rabbits after subcutaneous administration, nevertheless, it is a large dose and may have been more effective, given as it was, intracardially.

The events which are observed to follow the injection of large amounts of protamine into rabbits and mice, together with the pathologic findings, probably are not specific, resembling in many respects those seen after the parenteral introduction of various foreign substances, including proteins. To some extent our findings resemble those observed by Selye¹⁰ following sublethal doses of various drugs, as histamine, adrenalin, atropine, morphine, and formaldehyde. The "alarm reaction" produced is considered by Selye to represent simply the response of the organism to a damaging stimulus.

SUMMARY

1. The effect of the subcutaneous administration of large amounts of protamine (salmine) to 40 mice and 23 rabbits was studied.

2. The median lethal dose was found to be about 200 to 300 mg. per kilogram body weight.

3. The symptoms and signs produced by the injection of protamine consisted of respiratory distress, muscular rigidity, and opisthotonos, marked malaise, polyuria, fall in body temperature followed by a rise, and hyperglycemia. Dilatation of superficial blood vessels with some evidence of damage was noted.

4. Post-mortem examination of animals dying as the result of an injection of protamine showed signs of vascular damage with hemorrhages into the thymus, lungs, and kidneys. Other findings included infarcts in the kidneys, acute toxic changes in the kidney tubules, and focal necrosis of the liver.

5. It is concluded that the amount of protamine contained in insulin used in the treatment of diabetes represents so small a fraction of that found harmful to rabbits and mice that the possibility of its being a hazard is remote.

REFERENCES

1. Hagedorn, H. C., Jensen, B. N., Krarup, N. B., and Wodstrup, I.: Protamine Insulfate, *J. A. M. A.* 106: 177, 1936.
2. Thompson, W. H.: Die physiologische Wirkung der Protamine und ihrer Spaltungsprodukte, *Ztschr. f. physiol. Chem.* 29: 1, 1900.
3. Kossel, A.: The Protamines and Histones, New York, 1928, Longmans, Green & Co., p. 95.
4. Jappelli, A.: Sull'azione fisiologica di un solfato di protamino, *Boll. soc. ital. biol. sper.* 8: 778, 1933.
5. Walther, G., and Ammon, R.: Anaphylaktischer Shock nach Protamin, *Klin. Wchenschr.* 18: 288, 1939.
6. Folin, O. and Malmros, H.: An Improved Form of Folin's Micro Method for Blood Sugar Determinations, *J. Biol. Chem.* 83: 115, 1929.
7. Kern, R. A., and Langner, P. H., Jr.: Protamine and Allergy. I. Nature of the Local Reactions After Injections of Protamine Zinc Insulin. II. Induction of Sensitivity to Insulin by Injections of Protamine Zinc Insulin, *J. A. M. A.* 113: 198, 1939.
8. Taylor, A. E.: Chemical Studies in Cytolysis, *J. Biol. Chem.* 5: 311, 1908.
9. Wells, H. G.: The Chemical Aspects of Immunity, ed. 2, New York, 1929, Chemical Catalog Co., p. 28.
10. Selye, H.: Studies on Adaptation, *Endocrinology* 21: 169, 1937; see also Selye, H.: Über den Einfluss der Alarmreaktion und der Histaminbehandlung auf den Wasserstoffwechsel bei Nierenschädigung, *Klin. Wchenschr.* 17: 666, 1938.

PRELIMINARY OBSERVATIONS ON LOWERING OF BODY TEMPERATURES*

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WITH THE ASSISTANCE OF WILLIAM E. STIGERS

"PROFOUND oblivion midway between sleep and death" may be poetic eloquence, yet it expresses the intermediate stages of lethargy resulting from the associated efforts of sedation and refrigeration. The lowering of body temperature to approximately 20° F. in the dog by artificial means simulates natural hibernation in the upper strata of mammals.

The following report will endeavor to picture the physiological events occurring with metabolism at its apparently lowest ebb, and yet this ebb is compatible with life. To achieve this end, which others have suggested to be a palliative consideration for generalized cancer disease, a technique revolving around the simple idea of electrical refrigeration was employed.

In order to produce this lowered body temperature in a controlled manner, a cabinet cooled by electrical refrigeration was devised. This consisted of a rectangular wooden box insulated with several layers of cork. A swinging door, likewise insulated, was arranged to allow for free access to the interior, which measured 24 by 12 by 12 inches. The cabinet lay on its long side, facilitating such matters as laying the animal in a comfortable position and placing the necessary recording instruments. A metal shelf upon which the dog rested was set 6 inches from the floor of the cabinet, thus assuring sufficient byways and highways for the proper physics of refrigeration. From the ceiling of the box were suspended coils connected to the compressor, thereby completing the refrigerating system. In the ceiling and the floor were two one-inch vents, solving the problem of aeration for the animal. Emerging from the top of the cabinet, closely situated to the corners, were outlets just large enough to permit the exits of a 1/4 inch rubber tube for the intravenous anesthetic, a capillary tube from the rectal thermometer, a tube from the cabinet thermometer, a tube from the interior to the humidistat, a tube from the pneumograph to its manometer, a tube from the femoral artery to its pulsometer, and two metal tubes from the coils to the compressor unit.

Behind, and on the same stand with the cabinet, was placed the 1/4 H. P. compressor unit capable of 2,000 B.T.U.'s. Difluorodichloromethane was used for the refrigerant. This unit was capable of lowering the temperature of the interior of the cabinet (no animal) from 69° F. (room temperature) to 32° F. within eighty minutes.

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Anesthesia of the dogs was produced and maintained by continuous intravenous gravity (drop) method with a 0.5 per cent solution of pentothal sodium. This was given in all cases.

The dogs used were very much alike in type, weighing about 15 pounds. For obvious reasons, they were chosen for their short hair.

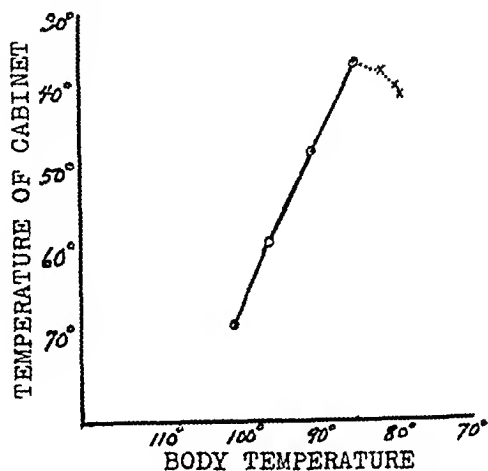


Chart 1.—Body temperature and cabinet temperature.

The actual experiment was conducted in the following manner: First, a $\frac{1}{2}$ inch No. 18 needle was inserted into the right cephalic vein and connected to the adapter of the intravenous apparatus. Sixty drops per minute induced the animal within ten minutes to the stage of insensibility. The dog was then placed upon the metal shelf so that it lay on its right side with its feet pointing outward. The pneumograph was connected around the lowest part of the chest. The pulsograph was taped to the left femoral artery. The thermometer was inserted deeply into the rectum, the tail was allowed to drop down between its legs and fastened in this fashion to secure a permanent position of the rectal thermometer. Everything was in readiness for the actual cooling after a normal set of readings of the pulse, respiration, temperature of the animal, temperature of the room, and the humidity was recorded.

Fifteen dogs were submitted to refrigeration under anesthesia. Two died in the early experiments due to an overdosage of the pentothal sodium. To study the effect of insulin and metrazol on the lowered body temperature, two other animals were sacrificed. Both injections were made when the body temperatures were below 80° F. The observations made thereof were extracurricular, and consequently, were not considered in this report. The remaining 11 dogs passed through the experiments and apparently returned to a normal condition. Two days after completion of the experiments they were observed to be in a normal state of health.

The observations considered in this report are based on the average of all the findings in these experiments, since variations from the average are negligible. For the sake of consistency, the same assistants recorded all the data. No untoward symptoms were encountered. No complications arose.

After the cooling apparatus was put into operation, readings were taken every fifteen minutes, however, now supplemented by the cabinet temperature.

Chart 1 contains changes of body temperature as compared with the cabinet temperature. From an apparent normal body temperature of 100.8°F. , the temperature dropped continuously with refrigeration. The lowest recorded body temperature was 79.5°F. whereas, at the same time, the cabinet temperature was 44°F. It was interesting to note that the cabinet temperature had to drop to 32°F. to make the "holdover" to carry on the lowering process. At this point the compressor was stopped, and as was expected, the body temperature gradually rose along with the cabinet temperature.

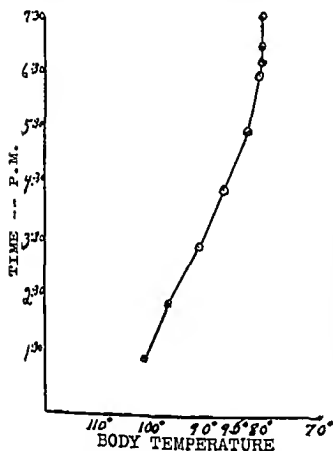


Chart 2.

Chart 2.—Time for body temperature to reach lowest point

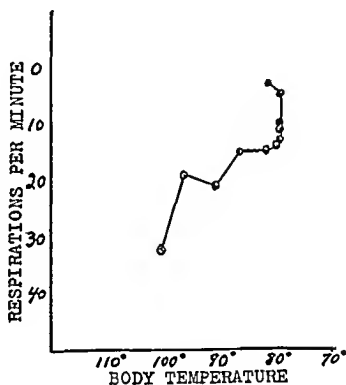


Chart 3

Chart 3.—Respiration and body temperature

Chart 2 elaborates the changes of body temperature as compared with the time required to make these changes. The $\frac{1}{4}$ H.P. compressor unit required six hours to lower the body temperature from normal to 79.5°F. This time element is striking as compared to the more than eleven hours necessitated for the body temperature to return to normal.

Chart 3 illustrates that respiration which decreased with the lowering of the body temperature was a more sensitive physiologic sign for judging the dog's condition than the pulse. Unlike Charts 1 and 2, Chart 3 is marked by the absence of a "straight" curve by being spotted with a number of fluctuations. The significance lies in the fact that respirations decreased to three per minute with the body temperature at 79.5°F.

Chart 4 compares the pulse changes with those of the body temperature. The former usually dropped along with the latter. When the body temperature registered 79.5°F. , the number of recorded pulsations per minute was 60.

However, the changes of the pulse rate did not occur as acutely as did the respiratory rate.

Chart 5, probably not as significant as the other charts, demonstrates the changes in humidity of the cabinet as related to the body temperature. The humidity rose as the body temperature fell.

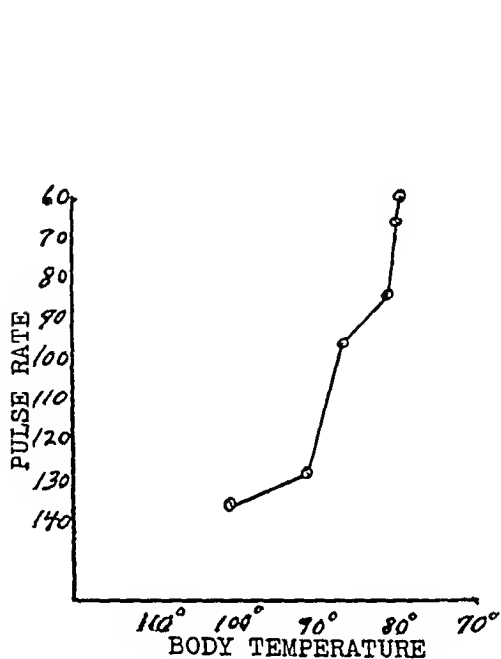


Chart 4.

Chart 4.—Pulse rate and body temperature.

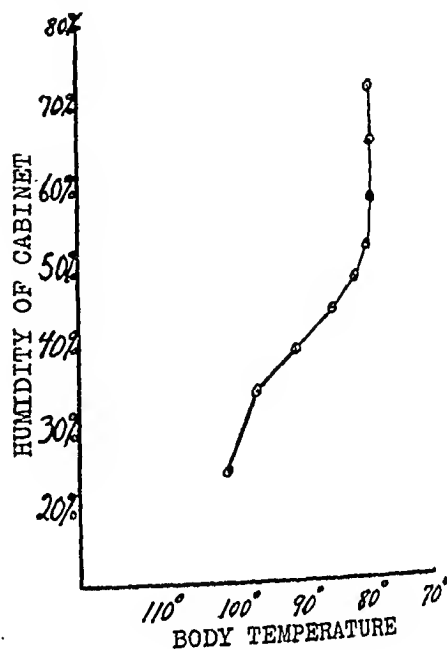


Chart 5.

Chart 5.—Humidity of cabinet and body temperature.

SUMMARY

The convenient method of lowering body temperature of dogs by the use of an electrical refrigeration unit has been described. The physiologic changes observed are:

1. The body temperature dropped to 79.5° F. when the interior cabinet temperature was 44° F.
 2. The time required for the above was six hours, almost half the time it took the dog to return to a normal temperature.
 3. The respiration dropped to three per minute when the rectal temperature read 79.5° F. The respiratory rate was a more reliable indication of the condition of the dog than was the pulse rate.
 4. The pulse rate dropped to 60 per minute as the body temperature dropped to 79.5° F.
 5. The humidity of the interior of the cabinet was 72 per cent with the body temperature at its lowest, 79.5° F. This reading became lower as the body temperature increased. The humidity may have been significant as a prophylaxis for pneumonia more than for its physiologic importance.
- Detailed deductions have been withheld pending completion of experiments on diseased dogs.

ALLERGIC PNEUMONIA*

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THE importance of the lung as a shock organ in allergic disease is now a commonplace. That true bronchial asthma is a manifestation due to the reaction of hypersensitiveness is universally accepted. In this condition the allergic reaction is confined to the bronchial tree.

A consideration of allergic pneumonia necessitates an entirely different concept. That an allergic response, including the *interstitial pulmonary* tissues, possibly is involved in the production of typical lobar pneumonia (pneumococcal) as well as certain types of pulmonary tuberculosis has long been considered.

However, an extensive pneumonitis on the basis of allergic response to agents other than those of infection is a relatively new and somewhat startling concept; namely, that the interstitial tissue of the lung is capable of sensitization and of reaction to foreign substances. That this may possibly be the case, however, is suggested by numerous reports. Loeffler¹ in 1932 described a disease to which he gave the name, "Flüchtige Sucedan-Infiltrate (mit Eosinophilie)." By 1936 Loeffler² had collected a total of 51 cases, characterized by infiltrative processes in the lung (demonstrated by x-ray), of leucocytosis and eosinophilia. In many instances other symptoms were few or lacking entirely, such cases being discovered through the procedure of group examination for tuberculosis. Reports of a similar condition include those of Busche,³ Wild,⁴ Engle,⁵ and Leitner.⁶ Gravesen⁷ in 1938 reported a case strikingly similar to the one presented in the following report, and suggested an allergic etiology. His patient, like the following, was receiving prontosil medication.

CASE REPORT

History.—S. L., a white male, aged 22 years, a law student at the University of Minnesota, admitted to the hospital January 15, 1939, complained of (1) frequent loose stools, (2) abdominal tenderness, (3) headache, (4) chills and sweats. The history of previous illness was unimportant except in relationship to the present complaints. In October, 1936, while he was in another city, he had a sudden onset of diarrhea, his stools containing bright red blood and mucus, for which he was hospitalized about six weeks. He returned to school in January, 1937. He had occasional recurrences of loose stools, but none were severe prior to the episode responsible for this admission.

Examination.—His temperature on admission was 100.6° F., pulse rate 106, respiration 22. Nutrition was fair. There was moderate abdominal distention with left lower quadrant tenderness. Physical examination otherwise showed nothing remarkable. Proctoscopic examination

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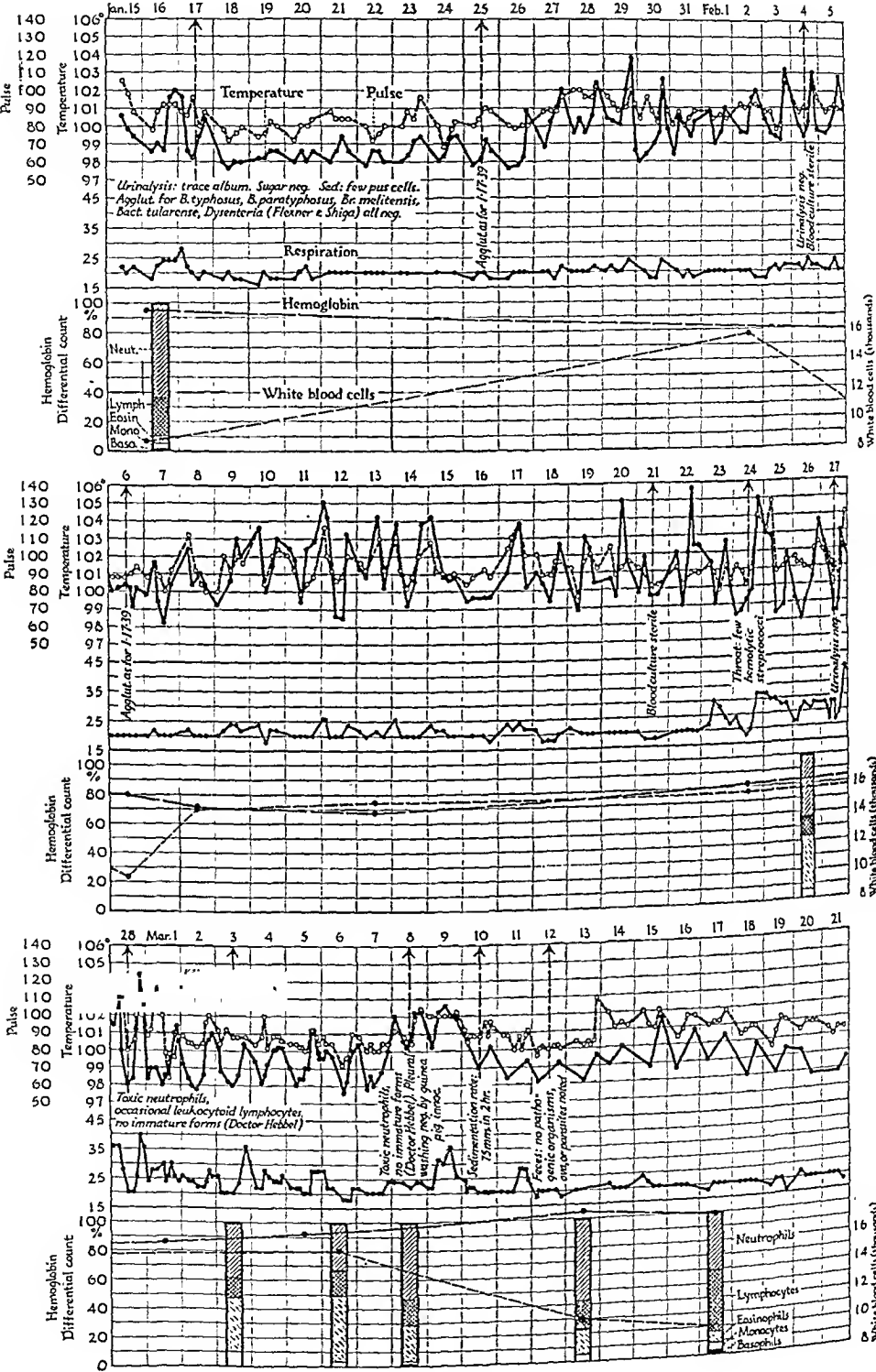


Chart 1.

by Dr. H. W. Christianson showed the bowel mucosa studded with pin-point ulcers which bled easily. Treatment prescribed by the proctologist included prontosil by mouth and a streptococcus vaccine parenterally. Prontosil was administered in 15 gr. doses every six hours (60 gr. daily) from January 25 to March 6, inclusive.

Clinical Course.—The illness with which this report is primarily concerned is considered to have started with a recrudescence of fever without other obvious new developments, and at a time when the colitis was showing marked improvement. The temperature was moderately elevated on admission but became normal two days later. During the succeeding week slight elevations of temperature occurred not exceeding 99.4° F. On January 25, the tenth day following admission, prontosil medication was initiated, with two 15 gr. doses at 6 p.m. and 10 p.m., respectively. On succeeding days the patient received four 15 gr. doses at six-hour intervals. On the evening of the eleventh hospital day (first full day of prontosil medication) the temperature rose to 100.8° F., the following day to 102° F., and on the next day to 103.8° F. Chilly sensations were mentioned, and nonproductive cough developed. No abnormal signs were noted on repeated physical examinations. X-ray of the chest on January 31 (seventeenth hospital day) showed marked thickening of the pleura, especially over the left apex, and diffuse increase in the bronchovascular markings, especially in the left lower lobe (Fig. 1).

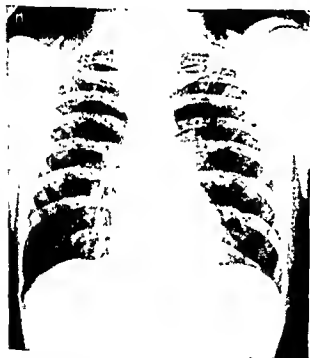


Fig. 1.



Fig. 2.

Fig. 1.—Seventh day of prontosil administration (Jan. 31, 1939).

Fig. 2.—Fourteenth day of prontosil administration (Feb. 7, 1939)

Physical signs continued to be absent until February 7, when a few fine crepitant râles were noted in the left axilla without other physical signs. X-ray at this time showed multiple areas of abnormal density throughout the left lower lobe and suggested a patchy bronchopneumonia (Fig. 2). Two weeks later x-ray films showed the left lung almost entirely involved and the appearance of an area of increased density on the right (Fig. 3). Crepitant and medium râles were heard over the areas involved without appreciable alteration of resonance, or breath sounds. A pleuropericardial friction rub appeared, accompanied by severe precordial pain; later there was pleural friction. These signs lasted only a few days and there was no effusion. Temperature of an irregular type at moderate to high levels prevailed throughout forty days (Chart 1). Rigors occurred during the height of the process with subsequent peaks of temperature, reaching 105° F., followed by profuse sweating. Cyanosis was not noted and the degree of prostration was so slight as to be indeed remarkable. Dyspnea, paroxysmal in type, was a complaint during a few days at the height of the disease, without obvious change in the physical signs and without cyanosis, but it was relieved in the oxygen tent. Prontosil was discontinued on March 7 after forty days of ad-

ministration, and improvement rapidly ensued. A vaccine which had been given had been discontinued three weeks earlier. The physical signs in the chest cleared and x-ray films on March 25 (fifty-nine days after the onset of the pulmonary symptoms) revealed only a moderate increase in the bronchovascular markings.

On the fifty-eighth hospital day the patient complained of pain in the left groin unaccompanied by obvious physical signs. Femoral phlebitis of low grade was suspected, and this diagnosis became obvious with the development of moderate swelling of the left leg only after the patient was allowed to get up about three weeks later. Aside from this condition, the patient was apparently well at time of discharge, April 4, seventy-three days after admission.



Fig. 3.



Fig. 4.

Fig. 3.—Twenty-first day of prontosil administration (Feb. 14, 1939).

Fig. 4.—Forty-second day of prontosil administration and date of discontinuance (March 7, 1939).

There was no further contact with the patient until the following January, when he re-entered school at the University of Minnesota. At this time the following additional history was obtained: During July the patient took neoprontosil for a few days without apparent reason other than "general principles" (to use his own words) and very shortly developed fever and symptoms of bronchitis (?). A physician was called; he noted râles without other findings. With discontinuance of prontosil the symptoms subsided promptly. No x-ray examination was done at that time.

The general physical examination on January 15, 1940, exactly one year following the hospital admission, was essentially negative. The Mantoux test was negative to 1 mg. of old tuberculin. The x-ray of the chest was also negative (Fig. 6).

Laboratory.—The urine was negative on admission and throughout. The blood on admission showed 8,800 leucocytes and hemoglobin content 95 per cent. The differential count showed polymorphonuclear leucocytes 64 per cent, lymphocytes 26 per cent, eosinophiles 5 per cent, monocytes 4 per cent, and basophiles 1 per cent. Two weeks later the leucocytes had increased to 15,800 and continued around that level to March 6. (See Chart 1 for results of a series of blood counts.) Differential counts were not done after admission until February 26, when an eosinophile count of 33 per cent was discovered. The eosinophiles increased to 46 per cent on March 7 (the date prontosil was discontinued), and then rapidly diminished to 7 per cent ten days later. Blood cultures were repeatedly negative. Agglutination tests on admission, and at two weeks and four weeks later, were negative for *Eberthella typhi*, *Salmonella paratyphi*; and related organisms, *Shigella dysenteriae*, *Brucella melitensis*, and *Pasteurella tularensis*. Sulfanilamide determinations showed a low concentration, consistently only a trace. The leucocyte count January 15, 1940, one year following admission, was 6,100, with 1 per cent eosinophiles. Stool examinations were repeatedly negative for *Entamoeba histolytica* and other animal parasites or ova.

Sputum was never present. Throat culture revealed a few *B. hemolytic streptococci* on one occasion.

The Mantoux test was negative on admission and shortly before discharge.

Culture and guinea pig inoculations with stomach washings were negative for *Mycobacterium tuberculosis*.

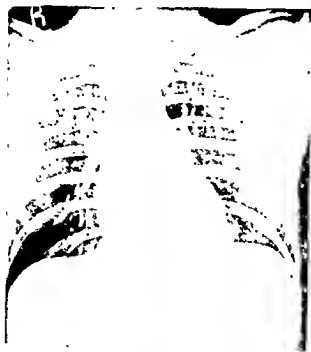


Fig. 5.



Fig. 6.

Fig. 5.—Eighteen days following discontinuance of prontosil (March 25, 1939)

Fig. 6.—One year following hospital admission (Jan 15, 1940).

DISCUSSION

The occurrence of an unusual type of pneumonia in which massive x-ray findings stood in decided contrast with the slight degree of prostration of the patient, as well as the absence of expected physical findings, had challenged our attention even before high-grade eosinophilia was demonstrated. Eosinophilia has long been recognized as a concomitant of the allergic response. We do not fail to recognize the fact that high-grade eosinophilia is also present in other conditions, occasionally in infections. We feel that such conditions as commonly are associated with eosinophilia were satisfactorily ruled out. Moreover, we do not believe that the pneumonia observed in our patient resulted from microbial invasion. Numerous clinicians of extensive experience were puzzled by it, and bacteriologic methods failed to demonstrate an etiologic agent. Granted that usual bacteriologic methods would have failed in the discovery of a virus as the etiologic agent, clinically it was not virus pneumonia, it did not occur in the course of an epidemic, nor was there any evidence of communicability.

The fever in this case is consistent with a diagnosis of allergic pneumonia, for fever is probably the most frequent symptom of drug allergy, and furthermore, has been frequently recorded in connection with allergy to sulfanilamide. The importance of the recognition of drug allergy as a cause for fever during the treatment of a disease which is in itself accompanied by fever is obvious. The sulfanilamide exhibited in this case was a treatment for ulcerative colitis

and not under the direction of the essayists. Consent to its discontinuance resulted in coincident prompt and complete recovery. The recurrence of similar symptomatology several months later, coincident with a further trial of prontosil, was not, unfortunately, under our own observation. Nevertheless, the history strongly suggests a repetition of the same pulmonary changes we observed. The shorter duration is in keeping with a shorter period of administration of the drug.

SUMMARY

A case of massive, migrating, atypical pneumonia of unusual duration, accompanied by high fever grossly disproportional to the degree of prostration, and a marked eosinophilia, is presented. The condition developed within a few days after initial prontosil administration, continued concomitantly with the use of this drug for six weeks, and cleared up rapidly coincident with its discontinuance. A recurrence of similar pulmonary symptoms not observed by the essayists is reported by the patient following a subsequent trial of prontosil.

We are indebted to Dr. Wesley J. Spink for having called attention to many of the foreign references cited, and who also first suggested the possibility of allergic etiology.

REFERENCES

1. Loeffler, W.: Zur Differential-Diagnose der Lungeninfiltrate (mit Eosinophilie), Beitr. z. klin. Med. 368, 1932.
2. Loeffler, W.: Die Flüchtigen Lungeninfiltrate mit Eosinophilie, Schweiz. med. Wchnschr. 66: 1069, 1936.
3. Busche, H. J.: Flüchtige Lungeninfiltrate als Hyperergische Gewebsreaktionen, München. med. Wchnschr. 85: 90, 1938.
4. Wild, O., and Loertscher, M.: Zur Aetiologie der flüchtigen Lungeninfiltrierungen, Schweiz. Med. Wchnschr. 15: 829, 1934.
5. Engel, D.: Über eine eigenartige, anaphylaktische Erkrankung der Lunge, Beitr. z. Klin. d. Tuberk. 87: 239, 1935.
6. Leitner, J.: Über flüchtige Lungeninfiltrate, Ztschr. f. Tuberk. 66: 226, 1932.
7. Graveesen, Paul B.: Transitory Lung Infiltrations With Eosinophilia, Acta med. Scandinav. 96: 523, 1938.

EFFECT OF HISTAMINASE ON THE SHWARTZMAN PHENOMENON*

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IT HAS been postulated that histamine plays an important role in the production of the Shwartzman phenomenon.^{1, 2} This suggests that histaminase, the specific enzyme which inactivates histamine, might be effective in preventing or modifying the reaction.

The following experiments were carried out in an attempt to determine the effect of histaminase on the Shwartzman phenomenon.

An active extract of *B. proteus* organisms for producing the Shwartzman phenomenon was prepared by a method very similar to that described by Antopol,³ which was based on procedures of Furth and Landsteiner⁴ and Levine and Frisch.⁵

In a preliminary experiment 8 rabbits were each given a preparatory dose of 2.5 mg. of the *B. proteus* extract and a provocative or reacting dose of 0.5

*From the Western Pennsylvania Hospital Institute of Pathology, Pittsburgh, Ralph R. Mellon, M.D., Director.

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mg. Four of these animals were pretreated with histaminase.* This histaminase was standardized by the Winthrop Chemical Co., 1 unit being the amount which will inactivate 1 mg. of histamine hydrochloride. The dosages administered orally and subcutaneously varied from 6 to 12 units per kilogram and correspond to six to twelve times the daily oral dose advocated for clinical use by the Winthrop Chemical Co. The intravenous dosages varied from 4 to 30 units per kilogram and correspond to one-third to two and one-half times the doses used by Karady and Browne⁶ on guinea pigs in histamine and anaphylactic shock. The results, as indicated in Table I, Series 1, showed no effect of the histaminase on the skin reaction.

As a result of numerous titrations, it was found that 0.1 mg. of the extract was effective as a preparatory dose, yet produced no gross lesion itself; and that 0.05 mg. as a provocative dose was minimal for eliciting the skin reaction in a high percentage of the animals tested.

Three more series, totaling 34 animals, were given these minimal doses of bacterial extract, and half of each series was pretreated with histaminase. It is seen from the results in Table I that again the histaminase had no striking effect.

TABLE I

RESULTS OF SHWARTZMAN REACTION WITH AND WITHOUT PRETREATMENT WITH HISTAMINASE

	SERIES 1		SERIES 2		SERIES 3		SERIES 4	
	NUMBER HISTA- MINASE RABBITS*	NUMBER CONTROL RABBITS	NUMBER HISTA- MINASE RABBITS†	NUMBER CONTROL RABBITS	NUMBER HISTA- MINASE RABBITS‡	NUMBER CONTROL RABBITS	NUMBER HISTA- MINASE RABBITS§	NUMBER CONTROL RABBITS
Died#	1	1			1			
++++	3	3						
+++			6	5	2	5	2	2
++			1	1	1		1	1
+							1	1
0			1	2	1			

* Death occurring shortly after provocative injection of extract

++++ Hemorrhagic skin reactions more than 4 cm. in diameter.

+++ Hemorrhagic reactions 2 to 4 cm. in diameter.

++ Reactions less than 2 cm. in diameter.

+ Very slight reactions or merely hyperemia.

0 No reaction.

*Enteric-coated histaminase capsules were given each rabbit as follows: 3 units orally five hours before the preparatory injection of extract, 3 units orally two hours after this injection; 3 units orally plus 3 units ground up with 5 c.c. of normal saline injected subcutaneously in the back four hours before the provocative injection, and 3 units subcutaneously immediately after this injection of extract. The suspensions of histaminase for injection were freshly prepared a few minutes prior to use.

†Each rabbit received tablets of histaminase as follows: 10 units ground up with 2 c.c. of normal saline injected subcutaneously six hours before the preparatory injection of extract; a similar injection one hour after the preparatory injection, and the same dose repeated one hour before the provocative injection. The suspensions of histaminase were freshly prepared a few minutes prior to use.

‡Each rabbit received 10 units of histaminase (injectable) powder dissolved in 10 c.c. of saline and injected intravenously fifteen to thirty minutes before the provocative injection of extract.

§Each rabbit received the supernatant fluid from 75 units of histaminase tablets ground finely in a mortar with 10 c.c. of normal saline, centrifuged, and injected intravenously fifteen to thirty minutes before the provocative injection of extract.

Since it requires twenty-four hours for 1 unit of histaminase to inactivate 1 mg. of histamine in vitro, it may be anticipated that the Schwartzman reaction develops too rapidly for histaminase to inactivate any histamine that may be released. The report of Karady and Browne⁶ appears to indicate that histamin-

*A small portion of the histaminase used in these experiments was furnished by the Winthrop Chemical Co., Rensselaer, N. Y., and the remainder, prepared by the same concern, was purchased in open market.

ase acts rapidly in vivo. However, using the same technique and a larger group of animals, Knoll⁷ was unable to confirm their work on anaphylactic shock.

An alternative worthy of consideration is that histamine-like substances rather than histamine itself may be responsible in the Schwartzman reaction. In this case, of course, it would not be expected that histaminase would have any effect on the reaction.

It is also possible that the increase in histamine content of the skin in the Schwartzman phenomenon is secondary, and an effect rather than a cause.

SUMMARY AND CONCLUSIONS

Little or no effect on the Schwartzman phenomenon was noted by pretreatment with histaminase administered orally, subcutaneously, or intravenously in large doses. Either histamine plays little part in the phenomenon or histaminase does not inactivate sufficiently the histamine released.

REFERENCES

1. Gratia, A., and Linz, R.: Les phénomènes de Sanarelli et de Schwartzman ou l'allergie hémorragique, *Ann. Inst. Pasteur* 49: 131, 1932.
2. Rocha e Silva, M., and Bier, O.: Dosage de l'histamine dans les lésions hémorragiques chez des lapins soumis au phénomène de Schwartzman, *Compt. rend. Soc. de biol.* 129: 1140, 1938.
3. Antopol, W.: The Production of the Schwartzman Phenomenon by Means of Bacterial Extracts, *J. Infect. Dis.* 61: 331, 1937.
4. Furth, J., and Landsteiner, K.: Studies on the Precipitable Substances of Bacilli of the Salmonella Group, *J. Exper. Med.* 49: 727, 1929.
5. Levine, P., and Frisch, A. W.: On Specific Inhibition of Bacteriophage Action by Bacterial Extracts, *J. Exper. Med.* 59: 213, 1934.
6. Karady, S., and Browne, J. S. L.: Effect of Histaminase Treatment on Histamine and Anaphylactic Shock in Guinea Pigs, *J. Immunol.* 37: 463, 1939.
7. a. Knoll, A. F.: Ineffectiveness of Histaminase in Anaphylactic Shock in Guinea Pigs, *Proc. Soc. Exper. Biol. & Med.* 45: 606, 1940.
b. Knoll, A. F., and Beinhauer, L. G.: Experimental and Clinical Observations With Histaminase, *Arch. Dermat. & Syph.* 42: 896, 1940.

PAROXYSMAL NOCTURNAL HEMOGLOBINURIA WITH HEMOLYTIC ANEMIA (MARCHIAFAVA-MICHELI SYNDROME)*

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THE syndrome of paroxysmal nocturnal hemoglobinuria was recorded first by Marchiafava and Nazari in 1911.^{1, 2} In 1931 Micheli³ reviewed all the cases published and added one to the literature. Since then other cases have been recorded by Hamburger and Bernstein,⁴ Witts,⁵ Scott, Robb-Smith, and Seowen,⁶ Dacie, Israels, and Wilkinson,⁷ and Ham.⁸ These authors have described the clinical, pathologic, and laboratory features which have established nocturnal hemoglobinuria as a specific disease.

From a clinical consideration of this disease, certain essential features are recognized. In the cases reported, it occurred most frequently during the second and third decades of life and, following a protracted course, terminated in death.

*From the Division of Medicine, University of California Medical School, San Francisco. Supported by a grant from the Christine Breon Fund for Medical Research. The blood counts were done by Miss Alice McBride. Received for publication, May 20, 1940.

Hemoglobinuria was the predominant sign and occurred almost always at night, frequently accompanied by vague abdominal pains. The course of the illness was characterized by recurring attacks of fever which were usually intermittent, a predisposition to venous thrombosis and phlebitis, and a slight icteric tint to the sclerae and skin. By the use of suitable reagents, hemosiderin and hemoglobin could be demonstrated in the urine. In a few cases slight enlargement of the spleen was reported.

The blood changes in nocturnal hemoglobinuria are most striking. In the cases reported in the literature, between one and three million red blood cells per cubic millimeter were noted. In most instances the cells were slightly larger than normal, so that the anemia was usually macrocytic, in others, it was normocytic. The hemoglobin was reduced to between 18 and 50 per cent of normal; hyperchromia was noted in some cases and hypochromia in others. Whenever hemolysis had occurred recently, hypochromia and consequently a lower color index were observed. In general, therefore, there was a tendency to slight macrocytosis (M.C.V. 100 cubic microns) and hypochromia of the red blood cells (M.C.H.C. 27 per cent). In all instances the anemia was refractory to treatment. The resistance to hypotonic salt solution was within normal range, and an examination of so-called "wet preparations" failed to show the presence of spherocytes. The outstanding feature was a persistent reticulocytosis. Invariably there was a leucopenia with a relative lymphocytosis and a moderate thrombocytopenia. Examination of the sternal bone marrow by either puncture or biopsy showed hyperplasia of the erythropoietic elements.

The etiology of hemoglobinuria is somewhat obscure. From a study of the reports in the literature it was apparent that, in contrast to familial hemolytic icterus, a familial predisposition did not exist in patients with nocturnal hemoglobinuria. Dacie, Israels, and Wilkinson,⁷ and Ham,⁸ were able to show by experiments both in vivo and in vitro that increased carbon dioxide tension was of critical importance for the production of hemolysis.

The following case of nocturnal hemoglobinuria is presented first, because of the rarity of the condition and, secondly, to record the results of in vitro studies showing increased hemolysis of red blood cells in media at an acid pH. The patient had an illness of several years' duration characterized by anemia, vague abdominal pain, thrombophlebitis, and hemoglobinuria occurring at night.

REPORT OF A CASE

History.—A. J., an American woman, single, aged 43 years, was first admitted to the University of California Hospital on May 31, 1938. She complained of weakness and stated that she was known to have been pale and anemic for at least six years. Five years previous to her present entry she had suffered profuse uterine bleeding which was relieved by salpingophorectomy and hysterectomy. Despite the cessation of menses, however, she continued to be anemic, and on two occasions obtained temporary relief from transfusions. In 1935 she had an attack of phlebitis in the right leg which persisted for several months. She had a similar attack in August, 1937, and in November, 1937, the veins of the left leg became involved. At this time she first noticed that her skin had a slightly yellowish tinge and experienced vague abdominal pains which were most marked at night. She then noticed that the urine passed on arising in the morning was very dark. In December, 1937, her physician told her she had had a hemorrhage from her kidneys. This episode lasted about one week and recurred about a month later.

Physical Examination.—On entry to the hospital the patient was well developed, moderately well nourished, looked tired and pallid, and had slightly icteric sclerae. The ears,

nose, throat, and lymph nodes presented no remarkable abnormalities. The teeth were carious, but the gums were firm and showed no bleeding points. There were no signs of disease in the heart or pulmonary parenchyma. The blood pressure was 122 systolic and 68 diastolic. Neither the spleen nor the liver could be felt on palpation. There was slight pitting edema of the ankles.

Laboratory Data. Examination of the blood showed red blood cells 1,970,000 per cubic millimeter, hemoglobin 56 per cent (7.8 Gm. Sahli), and leucocytes 2,200 per cubic millimeter. The differential count showed polymorphonuclear leucocytes 61 per cent (29 per cent filamented, 32 per cent nonfilamented forms), eosinophiles 3 per cent, basophiles 2 per cent, and small lymphocytes 34 per cent. The reticulocytes were 3 per cent. The volume of packed cells was 25 c.c. per 100 c.c. of blood. The mean corpuscular volume was 126 cubic microns, the mean corpuscular hemoglobin was 39 micromicrograms, and the mean corpuscular hemoglobin concentration was 31 per cent. The color index was 1.39, the volume index 1.47, and the saturation index 0.93. The fragility of the red blood cells established with the usual hypotonic saline solutions was within normal range. The rate of sedimentation of the erythrocytes was 24 mm. in one hour (corrected Wintrobe method). The reactions to the Wassermann and Kahn tests of the blood were negative. The phenolsulphonphthalein excretion tests showed normal renal function. No abnormalities of the kidneys were observed in the retrograde pyelograms. The determination of liver function by using the rose bengal dye showed normal excretion. The total blood serum protein was 5.82 Gm. per 100 c.c., of which 3.54 Gm. was albumin and 2.28 Gm. was globulin; the ratio of albumin to globulin was 1.55. Absence of hydrochloric acid from the gastric secretion was demonstrated by parenteral injection of histamine. Roentgenograms of the gastrointestinal tract and of the long bones revealed no abnormalities.

Bone marrow removed from the sternum for biopsy was examined by the usual technique of Zenker's fixation and staining with hematoxylin and eosin. It showed a general hyperplasia of both erythropoiesis and myelopoiesis. Large numbers of cells containing pyknotic nuclei and hemoglobin, and an abundance of younger forms with basophilic cytoplasm and large, deeply stained nuclei were observed. These were considered to be normoblasts and erythroblasts. No megaloblasts were seen. Only a few myeloblasts were noted, but there were large numbers of cells ranging from the stage of myelocytes to the fully developed polymorphonuclear leucocytes. The diagnosis was moderate generalized hyperplasia of erythropoiesis and leucopoiesis.

Course of Illness.—Repeated examination of the blood showed only little change in the red blood cells and their hemoglobin content, but the reticulocytes varied in number from 3 to 10 per cent of the total count. Ten days after entry the patient was discharged from the hospital to her physician who was advised to give her liver extract potent in pernicious anemia by injection and large doses of iron by mouth. In spite of this treatment the anemia persisted.

Second Entry. On August 19, 1938, the patient was readmitted to the hospital complaining that she had secreted dark urine for ten days. She stated that, except for weakness, she had felt about as usual until the present episode, when she began having chilly sensations, vague abdominal pains, and a daily afternoon rise in temperature to 100° F. She passed large quantities of bright red urine, which upon analysis by her physician was reported to contain blood.

TABLE I

	1:30 A.M.	7 A.M.	9 A.M.	NOON	3 P.M.	9 A.M.
Hemoglobin	++	++	0	0	0	0
Bile	0	0	0	0	0	0
Urobilin	1:10	1:50	1:10	0	0	0

The physical examination was essentially the same as previously reported.

Laboratory Data. The urine showed no bile, albumin, sugar, or casts. Urobilinogen was noted in a concentration of 1:50 dilution units in specimens obtained at 7 A.M., and 1:10 in specimens obtained at noon. Hemoglobin was present in specimens of urine obtained at

1:30 A.M. and 7 A.M., but was absent in specimens obtained after 9 A.M. (see Table I). Because of the presence of the hemoglobin in the urine, the patient's illness was recognized as paroxysmal nocturnal hemoglobinuria of the Marchiafava-Micheli type.

Special Procedures. A set of serologic experiments was arranged similar to those performed by Dacie, Israels, and Wilkinson.⁷ Serum oxalated plasma and heparinized plasma were used as hemolytic media. Duplicate series were set up with serum and plasma of a normal person of the same blood grouping (Moss II).

1. The patient's red blood cells, after having been carefully washed with physiologic salt solution, were added to tubes containing the patient's serum and plasma, and to tubes containing serum and plasma obtained from a normal person. The normal person's red blood cells were added to a series of tubes containing similar media. All tubes were placed in a water bath at a temperature of 37° C. One-tenth cubic centimeter of packed red blood cells was added to 1.0 c.c. of plasma or serum in each instance. The results are summarized in Table II. It is to be noted that a slight amount of hemolysis occurred when the patient's cells were mixed with her own and with normal serum. There was no hemolysis of the normal cells in similar preparations.

TABLE II

SHOWING A MARKED DEGREE OF HEMOLYSIS OF THE PATIENT'S RED BLOOD CELLS WHEN THE MEDIA ARE SLIGHTLY ACIDIFIED

	PHYSIO- LOGIC SALT SOLUTION	PA- TIENT'S SERUM	PA- TIENT'S OXALATED PLASMA	PA- TIENT'S HEPARIN- IZED PLASMA	NORMAL SERUM	NORMAL OXALATED PLASMA	NORMAL HEPARIN- IZED PLASMA
Patient's cells	0	Slight	0	0	Slight	0	0
Normal cells	0	0	0	0	0	0	0
Patient's cells {CO ₂ in solution	0	+++	+	++	++	+	++
Normal cells {CO ₂ in solution	0	0	0	0	0	0	0
Patient's cells {chilled serum		0	0		0	0	
Normal cells {chilled serum		0	0		0	0	
Patient's cells {chilled serum CO ₂ in solution		+++			+++	+	
Normal cells {chilled serum CO ₂ in solution		0	0		0	0	

2. Duplicates of the above procedures were set up, and carbon dioxide in 5 per cent dilution was bubbled through the serum and plasma. The results are also summarized in Table II. It is to be noted that in the acidified media definite hemolysis occurred in all solutions containing the patient's cells. In the tubes containing plasma (of the normal person and the patient), which had been treated with sodium oxalate to prevent coagulation, hemolysis was less marked. The hemolysis which occurred in the tubes containing normal cells with the serum of the normal person and the patient was negligible.

3. To determine whether or not there was a positive Donath-Landsteiner reaction, the serum and oxalated plasma were chilled prior to the addition of the red blood cells and the carbon dioxide gas. It is apparent from the data recorded in Table II that hemolysis was not increased by the low temperature.

4. In another series of experiments serum was heated to 56° C. The cells of the patient and those of the normal person were added to this serum. No hemolysis occurred even after bubbling the carbon dioxide gas through the serum. The hemolytic activity of the serum was not restored by the addition of complement. These results are summarized in Table III. This demonstrated that evidently the lysin was thermolabile and that the previous reactions were not due to a complement-fixation mechanism.

TABLE III
HEMOLYTIC ACTIVITY OF THE SERUM IS DESTROYED WHEN HEATED TO 56° C.

	PA- TIENT'S SERUM + CO ₂	PA- TIENT'S SERUM HEATED 56° C.	PA- TIENT'S SERUM HEATED + CO ₂	PA- TIENT'S SERUM HEATED + COMPLE- MENT	PA- TIENT'S SERUM HEATED + COMPLE- MENT + CO ₂	NORMAL SERUM HEATED	NORMAL SERUM HEATED + CO ₂	NORMAL SERUM HEATED + COMPLE- MENT	NORMAL SERUM HEATED + COMPLE- MENT + CO ₂
Patient's cells	++++	0	0	0	0	0	0	0	0
Normal cells	0	0	0	0	0	0	0	0	0

Treatment.—Since in vitro experiments showed an increased hemolysis at an acid pH, it was thought that the administration of large amounts of alkali might favorably alter the lytic mechanism. Accordingly, the patient was given a sufficient amount of sodium bicarbonate (4.0 Gm. four to six times a day) to render the urine alkaline (pH 7.6). However, during this treatment the patient had more severe abdominal pain and hemoglobinuria than before.

The oral administration of large doses of iron, the parenteral administration of liver extract effective in the treatment of pernicious anemia, together with a high protein diet, autolyzed yeast, and a period of multiple transfusions failed to increase the production of hemoglobin or red blood cells.

DISCUSSION

The diagnosis of our patient presented a considerable problem. She had an anemia probably of hemolytic origin accompanied by slight icterus and reticulocytosis. The degree of the anemia, the macrocytosis, the leucopenia, and the slight thrombocytopenia were suggestive of pernicious anemia. However, the failure of the bone marrow to respond to liver therapy removed this possibility from further consideration. The resistance of the red blood cells to hypotonic salt solution within normal range, the negative family history, and the absence of an enlarged spleen excluded the possibility of hemolytic icterus of familial origin. Studies of the urine during the patient's first hospitalization were inadequate, but during the second hospitalization discovery of the nocturnal emission of hemoglobin led to the recognition of the anemia as the hemolytic type described by Marchiafava.¹

No adequate explanation can be offered for the abnormal hemolytic mechanism in this patient. Special procedures were carried out according to the methods described by Dacie, Israels, and Wilkinson,⁷ and Ham.⁸ Our results

were comparable to theirs. After adding the patient's washed red blood cells to her serum and to normal serum, a slight degree of hemolysis was observed. When the media were acidified with carbon dioxide, the hemolysis was greatly increased. When the serum was heated to 56° C., the hemolytic activity was destroyed. It seems apparent from these studies that the patient's red blood cells were sensitized to some lysin present in her own serum and in normal serum. The role that hydrogen-ion concentration of serum or plasma may play in the production of the lysin deserves further investigation.

SUMMARY

The clinical and laboratory findings in a patient with nocturnal hemoglobinuria are presented. The possible significance of increased hydrogen-ion concentration in the production of lysin is briefly discussed.

REFERENCES

1. Marchiafava, E., and Nazari, A.: *Policlinico* 18: 241, 1911.
2. Marchiafava, E.: *Anemia emolitica con emosiderinuria perpetua*, *Policlinico* (sez. med.) 35: 105, 1928; 38: 105, 1931.
3. Micheli, F.: *Anemia (splenomegalia) emolitica con emoglobinuria-emosiderinuria tipo Marchiafava*, *Haematologica I. Arch.* 12: 101, 1931.
4. Hamburger, L. P., and Bernstein, A.: *Chronic Hemolytic Anemia With Paroxysmal Nocturnal*, *Proc. Soc. Exp. Biol.* 192: 301, 1936.
5. Witte, L. J.: *Lancet* 2: 115, 1936.
6. Scott, R. B., and Wren, E. F.: *The Marchiafava-Micheli Syndrome of Nocturnal Haemoglobinuria With Haemolytic Anaemia*, *Quart. J. Med.* 7: 93, 1938.
7. Dacie, J. V., Israels, M. C. G., and Wilkinson, J. F.: *Paroxysmal Nocturnal Haemoglobinuria of the Marchiafava Type*, *Lancet* 1: 479, 1938.
8. Ham, T. H.: *Chronic Hemolytic Anemia With Paroxysmal Nocturnal Hemoglobinuria: A Study of the Mechanism of Hemolysis in Relation to Acid-Base Equilibrium*, *New England J. Med.* 217: 915, 1937.

Since this paper was written, the patient died (Feb. 19, 1940). The cause of death was reported to be due to cerebral thrombosis. The spleen was normal in size.

FALSE POSITIVE SEROLOGIC REACTIONS FOR SYPHILIS IN INFECTIOUS MONONUCLEOSIS*

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MANY diseases besides syphilis may be accompanied by positive serologic reactions ordinarily considered diagnostic of syphilis. In malaria, during the acute stage, there is very frequently a transiently positive Wassermann or flocculation reaction.¹ Trypanosomiasis, relapsing fever, and ratbite fever are sometimes attended by temporarily positive tests.² Some patients with lymphogranuloma venereum³ and with kala-azar^{4, 5} also show such reactions. Approximately 60 per cent of leprosy patients have positive serology; and in yaws positive complement fixation and flocculation reactions occur as regularly as in syphilis.⁶ Christie⁷ has recently emphasized that many infants born to Wassermann-positive syphilitic mothers have positive serology at birth. In most instances this disappears without treatment and, therefore,

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represents merely the passage of reagin through the placenta. Serologic tests, in various degrees of positivity, have also been reported following vaccination for variola,^{8,9} and in cases of malignant neoplasms, jaundice, hyperpyrexia, pulmonary tuberculosis, typhoid, typhus, scarlet fever, "herpes genitalia," gonorrhea, "angina," bronchitis, and most often in upper respiratory infections and lobar pneumonia.¹⁰⁻¹³ However, the following statement is made by no less an authority than Eagle⁶: "The statistical reports of the past 10 to 15 years make it reasonably certain that neither tuberculosis, diabetes, pregnancy, anesthesia, malignancy, jaundice, fever, nor hypercholesterolemia predisposes to false-positive reactions."

A small number of references to the occurrence of false positive serologic reactions in infectious mononucleosis have appeared in the literature of the past decade. Weber¹⁴ in 1930, and Weber and Bode¹⁵ in 1931, reported 3 such cases with positive Wassermann and Meinicke tests. Gooding¹⁶ in 1931 stated that 16 of 27 patients with infectious mononucleosis gave either a positive or a doubtful Wassermann reaction, and that those patients who showed a negative Wassermann test in most instances showed a positive Kahn test. He stated further that the Wassermann reaction returned to negative spontaneously six to eight weeks after the acute stage. Unfortunately, details concerning the positive tests and the return to negative are most meager, so that it is not possible to say exactly how many of these cases should actually be included. One positive serologic test followed by repeated negative tests should not entitle a case to be included in our discussion, since this may represent merely a technical or clerical error.

Hatz¹⁷ in 1938 published one clear-cut case with strongly positive transient Wassermann reactions and simultaneously negative Kline tests. The next year Saphir¹⁸ reported one case with strongly positive Wassermann and Kahn reactions on several occasions, which became negative within several weeks without antisyphilitic treatment. Also in 1939 Sadusk¹⁹ described a patient whose blood gave temporarily positive reactions with the Kahn and the cholesterolized Wassermann antigens, and negative reactions with the alcoholic antigen. This case was definite; but the author incidentally mentions 2 others which are doubtful, since in both instances "weakly positive" reactions, reported on only one occasion, were not confirmed.

Bernstein² published an excellent account in 1938 of 6 cases in which the Wassermann or the Eagle flocculation test, or both, became temporarily positive during the course of infectious mononucleosis. Two of these cases had previously been reported by him.²⁰ Of the 6 cases, one had a "doubtful" Eagle, a negative Wassermann, and no further tests. This case should, in my opinion, be excluded from such a series. In a recent monograph on infectious mononucleosis, Bernstein²¹ states that 8 cases with false positive serology have been observed by him at the Johns Hopkins Hospital, thus adding 2 new cases to those previously reported.² The cases described by Löbe and Rosenfeld,²² Radford and Rolleston,²³ Wawersig,²⁴ and Asahina²⁵ should all be left out of any tabulation of false positive serologic reactions in infectious mononucleosis for one of two reasons: either the diagnosis of infectious mononucleosis was not certain or syphilis was not definitely excluded.

The incidence of false positive serologic reactions in infectious mononucleosis is difficult to determine with any degree of accuracy. According to the combined series of Gooding,¹⁶ Saphir,¹⁸ Sadusk,¹⁹ Bernstein,²¹ and myself, there were 29 patients with false positive reactions out of 183 patients with infectious mononucleosis who had serologic tests for syphilis performed. This gives an incidence of 15.8 per cent. But if Gooding's series—which is so contrary to the experience of all other clinicians—were excluded, the incidence would be only 8.4 per cent.

In contrast to these figures are the unpublished observations of several careful investigators²⁶ who, although they have heard indirectly of 2 or 3 unpublished cases of false positive reactions in infectious mononucleosis, have not personally observed a single such case in a combined experience of between 150 and 250 serologically tested patients. The actual incidence of recognized false positive reactions would, therefore, seem to be between 2 and 10 per cent. But as Bernstein² has stated: "It should be emphasized that the evanescent character of these positive serological reactions mitigates against the likelihood of recognizing all the instances in which they occur. It is reasonable to assume, therefore, that if these serological tests were carried out more regularly and persistently, the incidence of positive reactions encountered would be considerably higher."

Among 82 proved cases of infectious mononucleosis that I have investigated during the past three years, there were 3 with false positive serology. Sixty-four of the 82 had at least one serologic test for syphilis performed during the acute stage of the illness, and 32 of these had such tests on 2 or more occasions. The diagnosis of infectious mononucleosis was established by the clinical course, typical blood smears, and sheep cell agglutination tests. Two of the patients were known to have congenital syphilis with negative serology following antisypilitic treatment. It is interesting to note that in neither case did the Wassermann reaction revert to positive during the course of infectious mononucleosis.

TABLE I
SEROLOGIC REACTIONS IN CASE 1

DAY OF ILLNESS	WASSERMANN (ALCOHOLIC)	WASSERMANN (CHOLESTEROL)	KLINE	SHEEP CELL AGGLUTINATION TITER
11	Negative	2+	Negative	1:32
22	1+	4+	Negative	----
29	Negative	2+	Negative	----
32	Negative	1+	Negative	----
42	Negative	Negative	Negative	1:448
150	----	----	----	1:112
360	----	Negative	----	1:14

CASE REPORTS

CASE 1.—The first patient with false positive serology was a male pharmacy student, aged 22 years, who had had high fever, several chills, severe frontal headache, profuse sweats, marked fatigue, and general malaise for ten days prior to admission. He was sent to the hospital with the tentative diagnosis of typhoid fever. On admission he was found to have a markedly congested pharynx, slight enlargement of the spleen, marked hyperesthesia of the skin over the abdomen, and tenderness in the left lower quadrant. There was considerable

enlargement of the axillary, inguinal, and epitrochlear lymph nodes, all of which were firm and very tender. There were also a few small nontender left posterior cervical and bilateral anterior cervical nodes. The blood smear showed 12 per cent polymorphonuclear leucocytes and 88 per cent lymphocytes. The total white blood cell count was 13,400; and the red blood cell count and hemoglobin were within normal limits. On the fourteenth day of illness the monocytes numbered 11 per cent, and in the fifth week 40 per cent. Agglutination tests for typhoid and paratyphoid A and B infections were negative on two occasions. The results of the other serologic tests are shown in Table I.

CASE 2.—A male interne, aged 25 years, was admitted after having had a sore throat, cough, chills, and fever for one day. Examination revealed only a congested nasal mucosa and pharynx. The initial diagnosis was gripe. By the sixth day of illness he had a severe cough, high fever, mild conjunctivitis, and slight enlargement of the posterior cervical lymph nodes bilaterally. A positive sheep cell agglutination test and characteristic blood smears soon established the diagnosis of infectious mononucleosis. On the fourth day of illness the white blood cells were 11,400; polymorphonuclear leucocytes were 78 per cent, of which 27 were immature; lymphocytes 10 per cent; monocytes 12 per cent. On the ninth day the white blood cells numbered 15,000; polymorphonuclear leucocytes were 73 per cent, of which 40 were immature; lymphocytes 7 per cent; monocytes 4 per cent; eosinophiles 3 per cent; abnormal cells of the mononuclear series 13 per cent. Eight weeks after the onset a smear showed the following: polymorphonuclear leucocytes 39 per cent; lymphocytes 8 per cent; monocytes 9 per cent; eosinophiles 2 per cent; abnormal cells of the mononuclear series 42 per cent. The second blood smear was suggestive of infectious mononucleosis; subsequent ones were typical. The results of the various serologic tests performed in four different laboratories (a, b, c, d) are shown in Table II.

TABLE II
SEROLOGIC REACTIONS IN CASE 2

DAY OF ILLNESS	WASSERMANN (ALCOHOLIC)	WASSERMANN (CHOLESTEROL)	KLINE	MEINICKE	EAGLE AND NEW EAGLE	SHEEP CELL AGGLUTINATION TITER
9	--	4 + (a)	--	Negative (b)	--	1:56 (b)
19	--	2 + (a)	--	Negative (b)	--	1:112 (b)
32	4 + (b)	4 + (a)	Negative (a)	Negative (b)	--	1:28 (b)
41	4 + (b)	4 + (a)	Negative (a)	Negative (b)	Negative (d)	1:28 (b)
	4 + (c)	3 + (c)	Negative (c)			
		18 U. Positive (d)	Doubtful (d)			
54	± (b)	4 + (a)	Negative (a)	Negative (b)	Negative (d)	--
	4 + (c)	Negative (b)	Negative (c)			
		± (c)				
		6 U. Positive (d)				
72	2-3 + (b)	Negative (a)	4 + (c)	Negative (b)	--	1:112 (b)
	1 + (c)	Negative (c)				
89	± (b)	Negative (a)	Negative (c)	Negative (b)	--	1:56 (b)
	Negative (c)	± (b)				
		Negative (c)				
103	Negative (c)	Negative (a)	Negative (a)	Negative (b)	Negative (d)	--
		Negative (b)	Negative (c)			
		Negative (c)	Negative (d)			
		Negative (d)				

CASE 3.—A male child, aged 2½ years, was seen in the pediatric clinic after having had fever and anorexia for one week. A pin-point vesicular eruption was present over the entire body; the tonsils were enlarged and congested; there was a generalized lymphadenopathy; and the temperature was 104.2° F. Varicella was diagnosed and the child was

sent home. Two weeks later he was admitted to the hospital, since the fever, marked fatigue, and anorexia had continued, and there were in addition occasional vomiting and enuresis. Examination revealed temperature 100.6° F., pulse rate 104, respirations 24. There was a left catarrhal otitis media, injected tonsils and pharynx, and generalized lymphadenopathy.

A Widal test, a tuberculin test, and several urine examinations were all negative. A blood count, done the day before admission, showed white blood cells 17,000, polymorphonuclear leucocytes 4 per cent and lymphocytes 96 per cent. The following day the figures were hemoglobin 88 per cent, red blood cells 4,400,000, white blood cells 4,200, polymorphonuclear leucocytes 50 per cent, and lymphocytes 50 per cent. Four days later the smear showed polymorphonuclear cells 10 per cent, lymphocytes 29 per cent, monocytes 16 per cent, eosinophiles 1 per cent, and abnormal cells of the mononuclear series 44 per cent. The serologic reactions reported by several laboratories are summarized in Table III, wherein the headings "Kline" and "Eagle" refer to the Kline diagnostic and the Eagle micro-flocculation tests.

TABLE III
SEROLOGIC REACTIONS IN CASE 3

DAY OF ILLNESS	WASSERMANN (ALCOHOLIC)	WASSERMANN (CHOLESTEROL)	KLINE	MEINICK	EAGLE	KAHN	SHEEP CELL AGGLUTINATION TITER
26	Negative (b) Negative (c)	4 + (a) Negative (c)	4 + (c) 3 + (e)	Negative (b)	--	--	1:28 (b) 1:32 (c) 1:14 (e)
29	Negative (c)	Negative (c)	2 + (e)	--	--	--	--
33	Negative (b) Negative (c)	3 + (a) Negative (c) Doubtful (d) Negative (e)	4 + (a) Negative (c) Doubtful (d) Negative (e)	Negative (b)	Doubtful (d)	Negative (c)	1:7 (e)
39	Negative (b) Negative (c)	Negative (c) Doubtful (d)	2 + (c) Doubtful (d) Negative (e)	Negative (b)	Positive (d)	Negative (c)	1:14 (e)
49	Negative (c)	1 + (a) Negative (c) Negative (d)	Negative (a) Negative (c) 1 + (e)	Negative (b)	Doubtful (d)	2 + (c)	1:7 (e) 1:112 (b)
63	Negative (c)	Negative (a) Negative (c)	Negative (c)	Negative (b)	--	--	1:28 (h)
97	Negative (c)	Negative (c)	1 + (c)	--	--	--	1:56 (h)

DISCUSSION

What is the significance of a positive Wassermann or flocculation reaction during the course of infectious mononucleosis? In the first place, the patient may have syphilis, in which case the tests will remain positive. Secondly, the positive report may represent a technical error, in which case all subsequent tests will be negative.²⁷ Thirdly, the result may be and usually is a biological false positive reaction. It may happen that a positive Wassermann report is obtained before the diagnosis of infectious mononucleosis has been definitely made or even considered. One should, therefore, observe the dictum of Bernstein: "Whenever, in the course of any disease a Wassermann, suspected of being falsely positive, is encountered, a Paul-Bunnell [sheep cell agglutination] test should be performed." Even if the latter is negative, the patient may still have infectious mononucleosis.²⁸

Krag and Lonberg,¹² on the basis of a very large serologic experience, state: "If the positive [Wassermann] reaction at the first retest is constant and a thorough clinical-anamnestic investigation is completely negative, nothing

quantitative relationship between the heterophile antibodies, which give rise to the Paul-Bunnell reaction, and the substances responsible for the positive complement-fixation test (Wassermann) or for the various flocculation tests (Kline, Kahn, Eagle, Meinicke, etc.). As the cases in this paper illustrate, the same serum may give positive reactions with some tests for syphilis and negative reactions with other tests; different laboratories using presumably the same test may get totally different results with the same serum; and a positive test may be negative on the next examination, whereas a test previously negative may become positive. False positive reactions do not seem to be more frequently encountered with one serologic test than with another (Table IV), except in so far as certain tests, such as the Wassermann, are more commonly employed.

What is the cause of the false positive serologic reactions for syphilis occasionally noted during the course of infectious mononucleosis? According to Eagle's authoritative book on the laboratory diagnosis of syphilis,¹ there is likely to be some effect of native amboceptor (antibody for sheep red blood cells) on the Wassermann test. "Whereas small amounts of native amboceptor tend to give negative Wassermann reactions because hemolysis is accelerated, large amounts of native amboceptor, such as those usually observed in infectious mononucleosis, tend to cause such massive agglutination of the red blood cells as to retard hemolysis and therefore give a false positive result."² However, the possibility of a false positive flocculation test (Kline, Kahn, Eagle, Meinicke, etc.) being due merely to native amboceptor is clearly excluded, since red blood cells are not employed in this type of test. "The sera giving false-positive reactions apparently contain some reagin-like factor which reacts with beef heart lipid in a manner indistinguishable from syphilitic serum."³

The etiologic agent in infectious mononucleosis is at present unknown. Perhaps the occurrence, even though rarely, of false positive serologic tests for syphilis may offer a clue. Bernstein² has suggested that "the causative agent in infectious mononucleosis may contain a substance having antigenic properties in common with the *Treponema pallidum*; or else this causative agent may liberate from the tissues of the patient a reagin-like material. The fact that the majority of the diseases which may be associated with a positive Wassermann are caused by protozoa [including spirochetes as protozoa] would suggest that the causative agent of infectious mononucleosis may likewise be a protozoön."

SUMMARY

1. A review of the literature of false positive serologic tests for syphilis during the course of infectious mononucleosis has been presented.
2. Three additional cases were observed among 64 patients who had Wassermann tests performed.
3. The suggestion is made that patients should not be treated for syphilis until serologic tests, suspected of being falsely positive, have had a sufficient chance—about twelve weeks—to revert to negative.
4. The significance of a positive Wassermann or similar reaction during the course of infectious mononucleosis has been discussed.

¹ I wish to express my sincere thanks to the following for their kind aid: Doctors Alan Bernstein, Israel Davidsohn, Harry Eagle, Stephen Gumpert, John Koopman, Perry Manheims, Leo Spiegel, and Annis Thomson.

REFERENCES

1. Kitchen, S. F., Webb, E. L., and Kupper, W. H.: The Influence of Malarial Infections on the Wassermann and Kahn Reactions, *J. A. M. A.* 112: 1443, 1939.
2. Bernstein, A.: False-Positive Wassermann Reactions in Infectious Mononucleosis, *Am. J. M. Sc.* 196: 79, 1938.
3. Ravaut, P., and Raheau, P.: Transitorily Positive Wassermann Reactions in Lymphogranuloma Venereum, *Ann. de dermat. et syph.* 3: 80, 1922.
4. Greval, S. D. S., Sen Gupta, P. C., and Das, B. C.: Latent Syphilis in the Tropics, *Indian M. Gaz.* 73: 385, 1938.
5. Greval, S. D. S., Sen Gupta, P. C., and Napier, L. E.: Serological Reactions in Kala-azar: Complement-Fixation, False Wassermann Reaction, and High Anti-Complimentary Titre, *Indian J. M. Research* 27: 181, 1939.
6. Eagle, Harry: *Laboratory Diagnosis of Syphilis*, St. Louis, 1937, C. V. Mosby Co.
7. Christie, A. U.: Diagnosis of Syphilis in New-Born Infants, *Am. J. Dis. Child.* 55: 979, 1938.
8. Barnard, R. D.: False Positive Blood Serological Tests for Syphilis Following Vaccination for Variola, *Illinois M. J.* 77: 78, 1940.
9. Bay, A. P., and Sankstone, M.D.: Effects of Vaccination on Wassermann Tests, *J. A. M. A.* 115: 475, 1940.
10. Kahn, R. L.: Are There Paradoxic Serologic Reactions in Syphilis? *Arch. Dermat. & Syph.* 39: 92, 1939.
11. Stryjecki, T.: On Non-Specific and Temporarily Positive Bordet-Wassermann Reactions, *Bull. internat. Acad. polon. d. sc. et d. lett., Cl. mèl.*, p. 115, Jan., 1938.
12. Krag, P., and Lohberg, A.: The Occurrence of Strong Unspecific Wassermann-Kahn Reactions, *Acta dermat. venerol.* 19: 612, 1938.
13. Clifton, W. M., and Heinz, M. O.: A Survey of Prenatal Syphilis in a Hospital for Sick Children, *J. A. M. A.* 114: 1731, 1940.
14. Weber, F. P.: Glandular Fever and Its Lymphotropic Blood Picture—Sometimes Without Abnormal Glandular Enlargement, *M. Press* 181: 65, 1930.
15. Weber, F. P., and Bode, O. B.: Contributions on Glandular Fever, *München. med. Wchenschr.* 78: 1598, 1931.
16. Gooding, S. E. F.: On Glandular Fever or Infective Mononucleosis, *Practitioner* 127: 468, 1931.
17. Hatz, B.: The Wassermann Reaction in Infectious Mononucleosis With Report of a Case With Unusual Clinical Features, *Am. J. Clin. Path.* 8: 39, 1938.
18. Saphir, W.: The Wassermann Reaction in Infectious Mononucleosis, *Am. J. Clin. Path.* 9: 306, 1939.
19. Sadusk, J. F., Jr.: Temporarily Positive Kahn and Wassermann Reactions in Infectious Mononucleosis; Report of a Case, *J. A. M. A.* 112: 1682, 1939.
20. Bernstein, A.: Antibody Response in Infectious Mononucleosis, *J. Clin. Investigation* 13: 419, 1934.
21. Bernstein, A.: Infectious Mononucleosis, *Medicine* 19: 85, 1940.
22. Löhle, H., and Rosenfeld, H.: On Monocytic Angina With Transitorily Seropositive Erythema Nodosum, *Dermat. Ztschr.* 53: 373, 1928.
23. Radford, M., and Rolleston, J. D.: Two Cases of Glandular Fever Simulating Typhus, *Lancet* 2: 18, 1930.
24. Wawersig, R.: On Non-Specific Positive Reactions for Syphilis in Monocytic Angina, *Med. Klin.* 33: 1737, 1937.
25. Asahina, K.: Positive Hanganutziu-Deicher Reaction in Endemic Fever in South Japan, *Nagoya J. M. Sc.* 11: 79, 1937.
26. Personal communications from Dr. Charles Lyght, Madison, Wis.; Dr. William Marr, Galveston, Texas; Dr. Israel Davidsohn, Chicago, Ill.; Dr. Peter Vogel, New York, N. Y.
27. Moore, J. E.: The Modern Treatment of Syphilis, Springfield, 1933, C. C. Thomas.
28. Paul, J. R.: Infectious Mononucleosis, *Bull. New York Acad. Med.* 15: 43, 1939.
29. Mohr, C. F., Padgett, P., and Moore, J. E.: Syphilis: Review of the Recent Literature, *Arch. Int. Med.* 66: 1112, 1940.
30. Biologic False Positive Serologic Tests for Syphilis, Editorial, *Ann. Int. Med.* 14: 171, 1940.
31. Moore, J. E.: A Suggested Method of Approach to the Recognition of the Biologic False Positive Serologic Test for Syphilis, *Bull. Genito-Infect. Dis.* 3: 1, 1940.
32. Kahn, R. L.: A Serologic Verification Test in the Diagnosis of Latent Syphilis, *Arch. Dermat. & Syph.* 41: 817, 1940.
33. Krag, P.: Transient Unspecific Strong Wassermann-Kahn Reactions, *Ugeskr. f. læger* 98: 855, 1936.
34. Eagle, Harry: Personal communication.

EXPERIMENTAL UTEROTUBAL INSUFFLATION*

FURTHER INVESTIGATIONS

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IN A PREVIOUS communication¹ there were described some elementary features of uterotubal physiology as determined by insufflation of gas according to the method of Rubin. Uterotubal kinetics are analogous to those of other smooth muscle organs in that they are affected by the autonomic nervous system.

The present experiments were undertaken to evaluate the influence exerted by the extrinsic nerves upon the Fallopian tubes and uteri of rabbits, as determined by means of transuterine insufflation of gas. The influence of induced ovulation was also studied.

The female rabbit has a double uterus ending in a single vagina. Each uterus and its tube lie at the free end of a thin, highly vascularized mesentery. The uterine and ovarian vessels form multiple arcades, and anastomose close to the free border of the mesentery. The extrinsic nerves to the pelvic genitals pass through the mesentery, lying either free or in close conjunction to the vessels. There is probably a dual, anatomical innervation with one group of nerves following the ovarian and the other the uterine vessels.²

METHOD

Young female rabbits, weighing 2.2 to 5.4 pounds, were insufflated by the technique previously described. The effect of nerve block as induced by infiltration of the uterine or tuboovarian mesentery with 2 per cent procaine hydrochloride was studied. Direct electrical stimulation of the mesentery was attempted, but the contractions of the smooth muscle in the mesentery itself rendered the resultant kymographic tracing difficult to interpret. Trausection of the spinal cord was found to be so shocking as to invalidate conclusions at this time. An attempt was made to divide the mesentery, leaving the blood supply intact, but the operation was difficult to perform without introducing mechanical factors and at the same time it was impossible to eliminate the presence of undivided nerve fibers traveling in the vascular sheaths.

EXPERIMENTAL FINDINGS

The features of the curve that are particularly emphasized are: (1) Primary pressure peak—level at which gas enters the abdominal cavity. (2) Plateau level—general level of pressure during insufflation. (3) Small waves of fluctuation in pressure during insufflation.

Nerve Block of the Uterine Mesentery.—In 11 animals 1.0 to 1.5 c.c. of procaine was injected throughout the uterine mesentery from the cervix to the uterine cornu. Four animals were observed without any systemic anesthesia,

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and in the other 7 efficient narcosis was elicited by intravenous nembutal. After a preliminary control insufflation and when a fairly constant plateau level of insufflation was observed, the procaine was injected. Changes in the nature of the curve appeared within three to sixty seconds after injection.

TABLE I

PLATEAU PRESSURE LEVEL OF CONTROL	PLATEAU LEVEL AFTER PROCAINE	FALL
mm. Hg	mm. Hg	mm. Hg
112	58	54
90	45	45
75	50	25
72	48	24
70	58	12
57	28	29
55	45	10
50	45	5
48	25	13
45	42	3
45	40	5

1. Effect upon plateau level of insufflation. This level fell in all 11 animals. The range of fall was between 3 and 54 mm. Hg, and the amount of fall was less as the control plateau level approached 45 mm. Hg (Figs. 1 and 2).

2. Effect upon the character of the small waves of pressure.

(a) In 7 animals there was no apparent change in the nature of the waves.

(b) In 2 animals after a fall in the plateau level the waves became deeper and more regular.

(c) In one animal after procaine block of the uterine mesentery the plateau level fell only 3 mm. Hg, but the small waves of pressure which had been observed in the control period disappeared completely. In another animal the plateau level fell from 72 to 48 mm. Hg, and all the waves similarly disappeared. In both of these animals reinsufflation was performed and all the waves reappeared (Fig. 3).

3. Effect upon the tracing gained through reinsufflation. This was performed in 7 animals.

(a) In all cases the primary peak at which gas entered the abdominal cavity was lower in the second insufflation obtained after blocking the uterine mesentery than in the control tracing. The arithmetic mean for the first tracing was 109 mm. Hg and for the second was 62 mm. Hg. This decrease is not to be attributed solely to procaine, for it has been shown that simple reinsufflation of itself lowers the primary pressure peak. (In a group of ten animals who were reinsufflated without procaine injection the mean for the primary peak of the first tracing was 130 mm. Hg and of the second 79 mm. Hg).

(b) In all cases the plateau level of the second insufflation was the same or slightly lower than that obtained under procaine.

(c) The small waves of pressure usually appeared more regularly and were deeper than in the previous insufflation.

4. Saline infiltration of uterine mesentery. In 3 animals saline solution in 1 to 1.5 c.c. amounts was injected into the uterine mesentery to rule out any mechanical effect of fluid distention. In 2 animals no change in the tracing

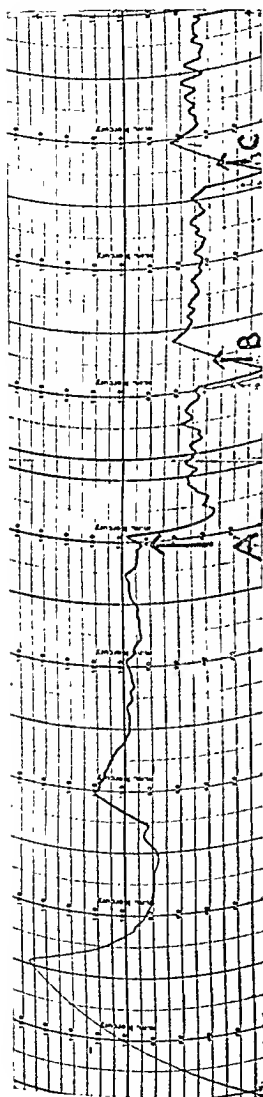


Fig. 1.—Effect of procaine injection of the uterine mesentery. Primary peak—174 mm. Hg. Plateau level—90 mm. Hg. At A, injection of 1 c.c. of 2 per cent procaine hydrochloride into the uterine mesentery. Note. Immediate drop to level of 13 mm. Hg and maintenance of level upon re-insufflation at B and C.



Fig. 2.—Effect of procaine injection of the uterine mesentery. Primary peak—92 mm. Hg. Plateau level—57 mm. Hg. At A, injection of 1 c.c. of 2 per cent procaine hydrochloride into the uterine mesentery. Note. Drop to level of 28 mm Hg and maintenance of level upon re-insufflation at B.

occurred. In one animal the plateau level fell 7 mm., but the character of the tracing remained the same. In all 3 cases procaine was then injected into the areas previously infiltrated with saline. In 2 of the 3 animals the plateau level was decreased about 15 mm. Hg (there was considerable dilution of the procaine by previously injected saline), and the reinsufflation curves were similar to those seen under procaine insufflation.

5. In one animal no gas entered the cavity, although a pressure of 200 mm. Hg was reached. The animal was reinsufflated and the uterine mesentery injected with procaine while the pressure was still rising. Gas entered to form a primary peak at a level of 175 mm. Hg and fell to a plateau of 70 mm. Hg. Another cubic centimeter of procaine was injected into the mesentery, and the level fell further to 55 mm. Hg. On reinsufflation the primary peak was at a level of 88 mm. Hg and the plateau was 60 mm. Hg.

TABLE II

CONTROL PRIMARY PEAK	PLATEAU	UNDER TUBOOVARIAN BLOCK	UNDER UTERINE BLOCK
mm. Hg	mm. Hg		
108	78	98, fell to 78	64
170	95	135, fell to 60	
124	63	45, reinsufflated to produce a primary of 90 and plateau of 45	30
168	80	Up to 100	45
152	52	Up to 58	To 35 and rose gradually to 53

TABLE III

RATE OF GAS FLOW IN C.C./MIN.	PRIMARY PRESSURE PEAKS IN MM. HG	
	OVULATING	NONOVULATING
11	140	108
15	148, 78	143
18	155, 105, 90	117
19		152, 142
20	158, 127, 54	
21	200, 50	200, 168, 168, 124, 119, 100
22		200, 100
24		168
25		115
27		183
28	128	80, 65
30	74	174
32		92
35	173	150
37		
40	100	174
42		88
50		

Nerve Block of the Tuboovarian Mesentery.—1. In 2 rabbits 1 c.c. of saline was injected into the tuboovarian mesentery and no change was observed in the tracing.

2. In a rabbit whose uterine mesentery had previously been injected with procaine to produce a slight fall in plateau level, injection of the tuboovarian mesentery produced no change.

3. In 5 experiments procaine was first injected into the tuboovarian mesentery. In 4 of these cases the plateau level rose slightly while in one case it fell. In 4 cases the uterine mesentery was then infiltrated with procaine and a fall was observed in the plateau level in all cases (Figs. 4 and 5)

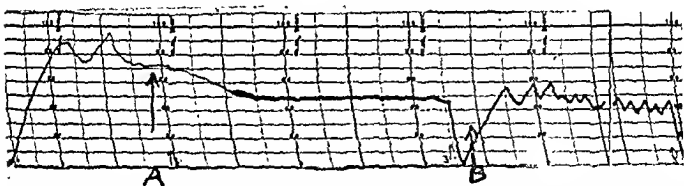


Fig. 3.—Effect of procaine injection of the uterine mesentery. Primary peak—88 mm. Hg. Plateau level—72 mm. Hg. At A, injection of 1 c.c. of 2 per cent procaine hydrochloride into the uterine mesentery. Note: Drop to level of 48 mm. Hg and disappearance of all waves on the tracing. On re-insufflation at B, the waves returned

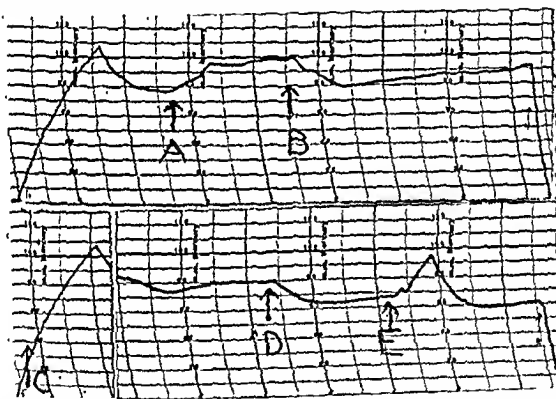


Fig. 4.—Effect of block of the tuboovarian mesentery followed by block of the uterine mesentery. Primary peak—108 mm. Hg. Plateau level—78 mm. Hg. At A, injection of 1 c.c. of procaine into the tuboovarian mesentery. At C, re-insufflated. At D, another cubic centimeter of procaine injected into the uterine mesentery. At E, another cubic centimeter of procaine injected into the tuboovarian mesentery. Note: Drop in levels on injecting the uterine mesentery and rise in levels upon injecting the tuboovarian mesentery.

Effect of Induced Ovulation.—In 15 animals ovulation was induced by the injection of human pregnancy urine. Ten cubic centimeters of urine were injected two days in succession, and on the third day insufflation was performed. The effect upon the primary pressure peaks was compared to that of 23 non-ovulating animals.

The arithmetic means for the two groups are 104 for the ovulating animals and 136 for the nonovulating. This difference is insignificant in view of the scattering.

DISCUSSION

We have demonstrated that definite changes are observed in the kymographic tracings after blocking the uterine mesentery with a local anesthetic.

The actual part played by the ovarian plexus as compared to the uterine, as well as the factors of the sympathetic as opposed to parasympathetic innervation, have not as yet been elucidated.

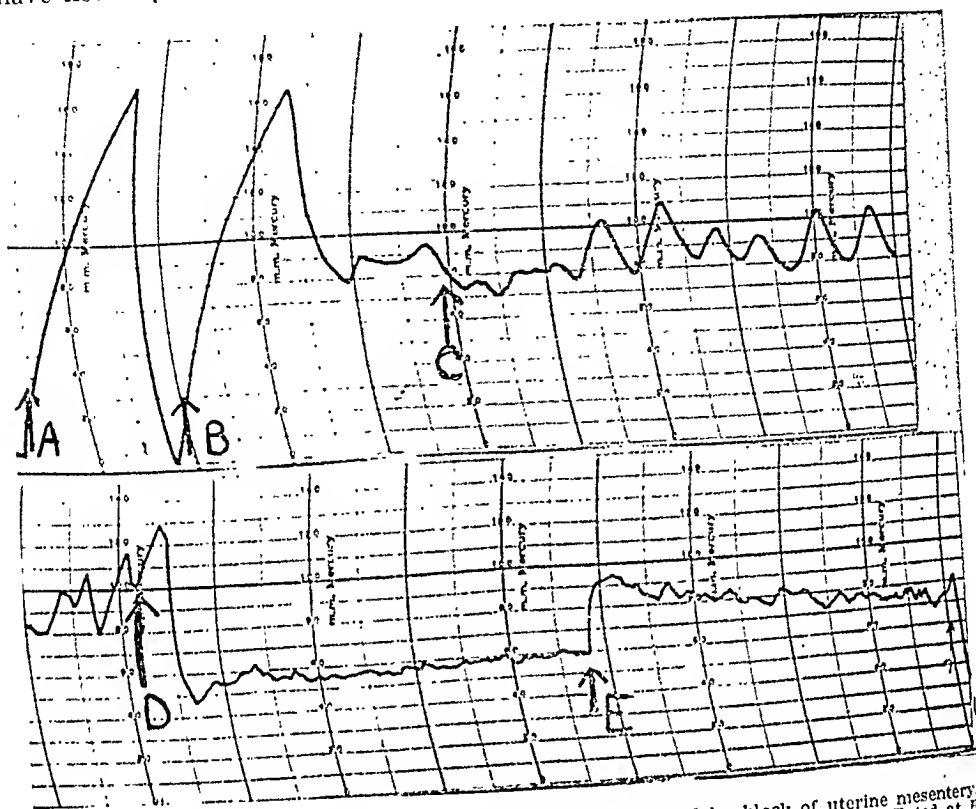


Fig. 5.—Effect of block of tuboovarian mesentery followed by block of uterine mesentery. The animal was insufflated at A with no gas passing into abdominal cavity. Reinsufflated at B at the same rate of gas flow. Primary peak—168 mm. Hg. Plateau level—80 mm. Hg. At C, 1 c.c. of procaine injected into the tuboovarian mesentery. At D, 1 c.c. of procaine injected into the uterine mesentery. At E, the rate of gas delivery was increased from 20 to 100 c.c. per minute. Note: Rise in level after injecting the tuboovarian mesentery and fall in level after injecting the uterine mesentery. On speeding the rate of gas delivery five times, the level rose 35 mm. Hg and gradually fell.

In a previous communication it was demonstrated that upon simple re-insufflation of rabbits both the primary peaks and the plateau levels fall to reach a constant level after about thirty minutes of insufflation. This particular level is promptly reached after the injection of procaine into the uterine mesentery. Furthermore, although an increase in gas rate increases the primary peak and the plateau levels,¹ upon continuing the insufflation the level falls to reach that obtained at slower rates of flow. This phenomenon has been observed in animals whose uterine mesentery has been blocked. Thus there seems to be a basic level of insufflation pressure, or plateau level, which is kept fairly constant by some intrinsic compensating mechanism, irrespective of time, nerve impulses, and delivery rate.

The obvious clinical applications of these findings are now being studied on patients, with particular reference to the case management of sterility and the relief of dysmenorrhea.

REFERENCES

1. Newman, H.: Experimental Utero-tubal Insufflation, *J. LAB. & CLIN. MED.* 26: 1129, 1941
2. Reynolds, S.: The Physiology of the Uterus, New York, 1939, Paul B. Hoeber, Inc.

NONFILAMENT-FILAMENT RATIO CHANGES FOLLOWING ADMINISTRATION OF AUTOGENOUS VACCINES TO PERSONS WITH LOW-GRADE CHRONIC ILLNESS

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IT HAS been stated that the ratio of immature to mature neutrophilic leucocytes parallels the activity of an infectious disease process¹ and the severity of symptoms,² and that clinical improvement in patients with low-grade chronic illness is accompanied by proportionate improvement in the immature-mature cell ratio.³ It has been suggested also that the ratio of immature to mature neutrophils might be used in the control of vaccine therapy.^{1a, 1b} The present study was made to determine whether there might be changes in the ratio of young to old neutrophils which would correlate with the subjective responses which sometimes follow the injection of autogenous vaccines.

MATERIAL AND METHODS

Observations were made on a group of persons with low-grade chronic illness in whom chronic infection was considered to be an important factor. Detailed analyses of this group as to clinical condition, sedimentation rate, and nonfilament-filament ratio,² and incidence of probable pathogenic streptococci and staphylococci⁴ have been reported. Autogenous vaccines prepared from organisms isolated and selected by methods previously described^{4, 5} were administered subcutaneously in varying dilutions to these persons. At times following injection of the diluted vaccines there were definite subjective responses which could be reproduced by reinjection of the same doses of vaccine at appropriate intervals.

Blood smears were made at frequent intervals, sometimes several times daily when the sequence of changes was rapid, from persons in whom definite subjective responses were noted. Differential blood counts were made, neutrophils being divided into nonfilamented and filamented types (or nonsegmented and segmented types, no distinction being made between the two methods⁶), according to the criterion of Haden,⁷ i.e., "neutrophils in which two or more lobes are united only by a filament of chromatin material are recorded as filamented cells, all others are classified as nonfilamented." The ratio of nonfilamented to filamented neutrophils (referred to as "nonfilament-filament ratio") was expressed in percentage; i.e., nonfilament-filament ratio =
$$\frac{\text{nonfilamented cells} \times 100}{\text{filamented cells}}.^2$$

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RESULTS

To a large extent the changes in the nonfilament-filament ratio paralleled subjective changes, i.e., when symptoms were increased the nonfilament-filament ratio was increased, and when symptoms were lessened the nonfilament-filament ratio was lessened.

Three general types of subjective response to vaccine injection were noted, with corresponding nonfilament-filament ratio changes:

Type I: In the first type there was a period of definite lessening or cessation of symptoms, accompanied by a feeling of well-being, lasting from a few days to a week or more.

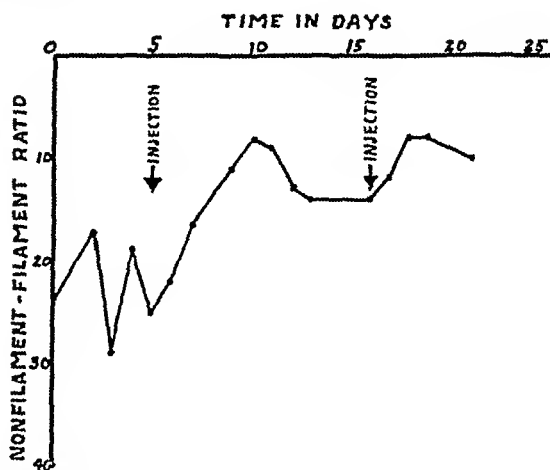


Fig. 1.—Variations in the nonfilament-filament ratio during a five-day control period and after two injections of vaccine which were followed by subjective improvement (type I response).

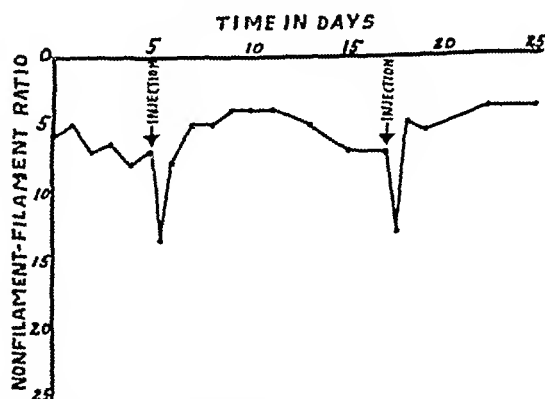


Fig. 2.—Variations in the nonfilament-filament ratio during a control period and after vaccine injections in a person who developed transient unfavorable symptoms (less than twenty-four hours) followed by definite improvement (type II response).

Type II: The second type of response was characterized by an initial period of increased intensity of illness, with malaise, depression, headache, or other general symptoms (the so-called negative phase), followed by a period of definite improvement. The negative phase lasted from a few hours to a few days, and the subsequent period of improvement from a few days to a week or more.

Type III: The third type was characterized by a prolonged negative phase, lasting a week or more, with little or no subsequent improvement. In this type

the unfavorable symptoms of the negative phase were usually milder in intensity and more delayed in onset than the unfavorable symptoms in type II responses.

In Figs. 1, 2, and 3 are shown typical examples of the nonfilament-filament ratio changes accompanying the different types of subjective response. In each instance there are included observations of the nonfilament-filament ratio made on each of the five days prior to the injection of vaccine, and the observations made subsequent to two injections.

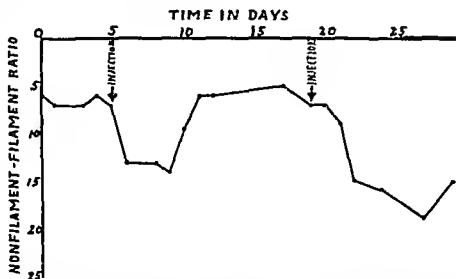


Fig. 3.—Variations in the nonfilament-filament ratio during a control period and following vaccine injections in a person who developed prolonged unfavorable reactions with very little subsequent improvement (type III response). Note that following the second injection there was a delay of more than a day in the onset of the unfavorable change in the nonfilament-filament ratio.

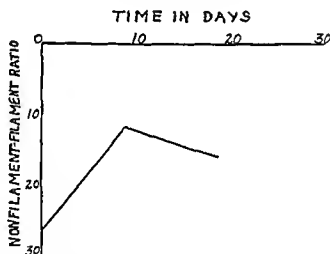


Fig. 4.—Composite graph based on the means of the nonfilament-filament ratio observations in 28 persons who improved following vaccine injection (type I response).

Fig. 4 is a composite graph based on the means of the nonfilament-filament ratio observations at the time of injection, at the time of greatest improvement, and at the time of final observation, of 28 persons who improved following vaccine injection (type I response). The mean nonfilament-filament ratios were 26.75, 11.6, and 16.0, respectively. The separate graphs of the 28 persons are shown in Figs. 4A, 4B, and 4C.

Fig. 5 is a composite graph similarly based on the means of the nonfilament-filament ratio observations of 12 persons who experienced preliminary unfavorable symptoms following injection. In these persons the unfavorable reaction, which lasted from one to five days, was followed by a period of definite improvement (type II response). The mean of the nonfilament-filament ratios at the time of injection was 18.4; at the time of greatest increase in symptoms, 30.0;

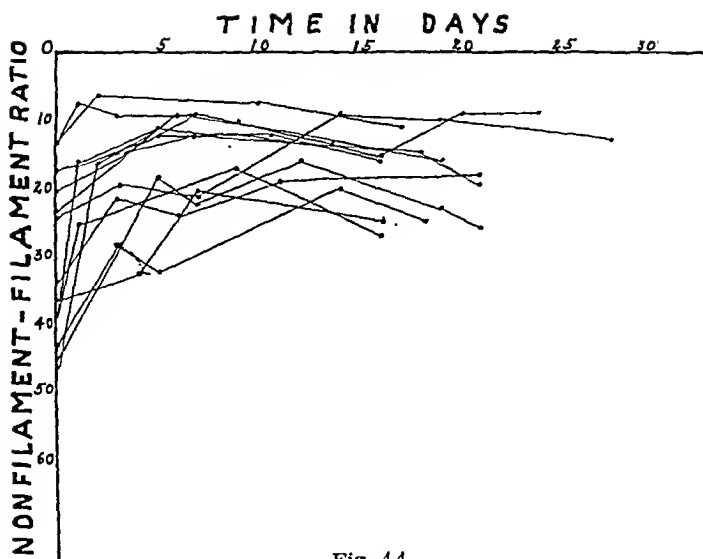


Fig. 4A.

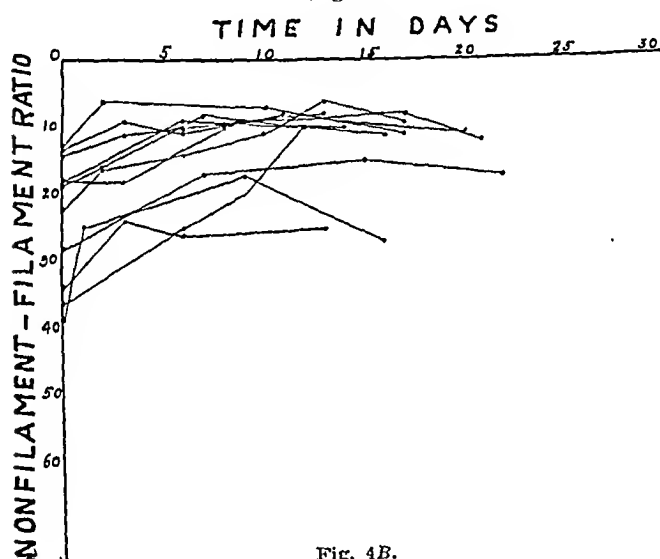


Fig. 4B.

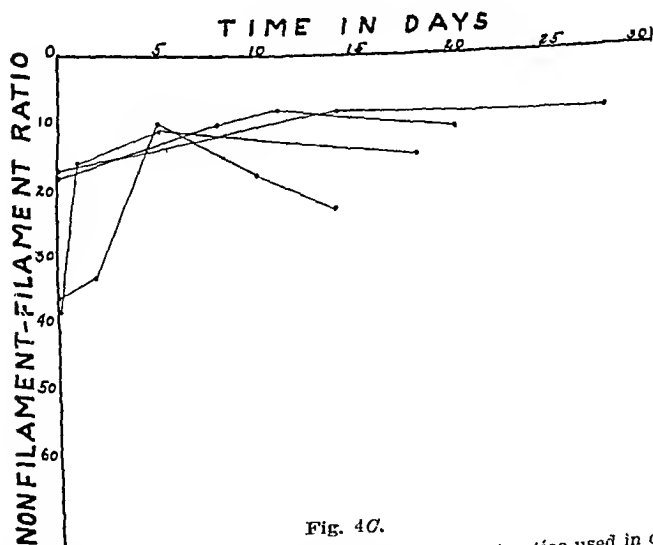


Fig. 4C.

Figs. 4A, 4B, and 4C.—Separate records of nonfilament-filament ratios used in computing Fig. 4.

at the time of greatest improvement, 10.5; and at the time of final observation, 14.3. The separate curves of nonfilament-filament ratio change in the 12 persons are shown in Fig. 5A.

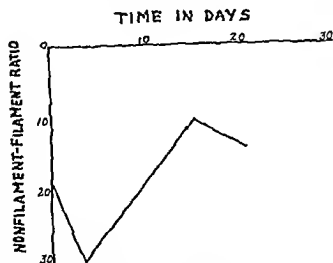


Fig. 5.—Composite graph based on the means of the nonfilament-filament ratio observations in 12 persons who, after vaccine infection, experienced preliminary unfavorable symptoms followed by definite improvement (type II response).

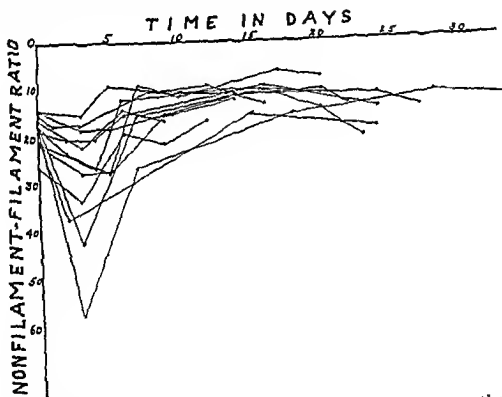


Fig. 5A.—Separate records of nonfilament-filament ratios used in computing Fig. 5.

Fig. 6 is a composite graph based on the means of the nonfilament-filament ratio observations of 8 persons in whom there was a prolonged unfavorable reaction (type III response). The mean of the individual nonfilament-filament ratios at the time of injection was 15.5; at the time of the most severe symptoms, 29.3; at the time of greatest improvement, 13.0; and at the time of final observation, 14.9. The separate graphs of the nonfilament-filament ratio changes of the 8 persons are shown in Fig. 6A. In this group of persons there was little, if any, subjective improvement following the prolonged unfavorable reaction, and the mean of the nonfilament-filament ratio observations at the time of greatest improvement was very little better than the mean of the original observations.

Providing there was no intercurrent illness, there were no significant non-filament-filament ratio variations observed in persons receiving no injections,

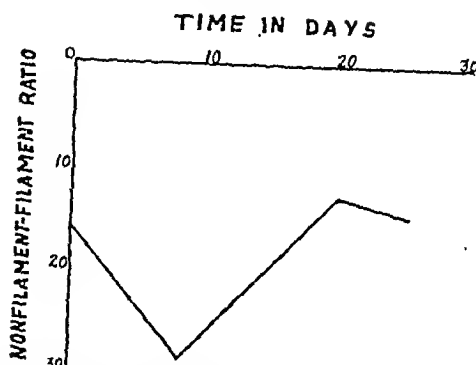


Fig. 6.—Composite graph representing the means of the nonfilament-filament ratio observations in 8 persons who experienced prolonged unfavorable reactions with little or no subsequent improvement (type III response) following vaccine injection.

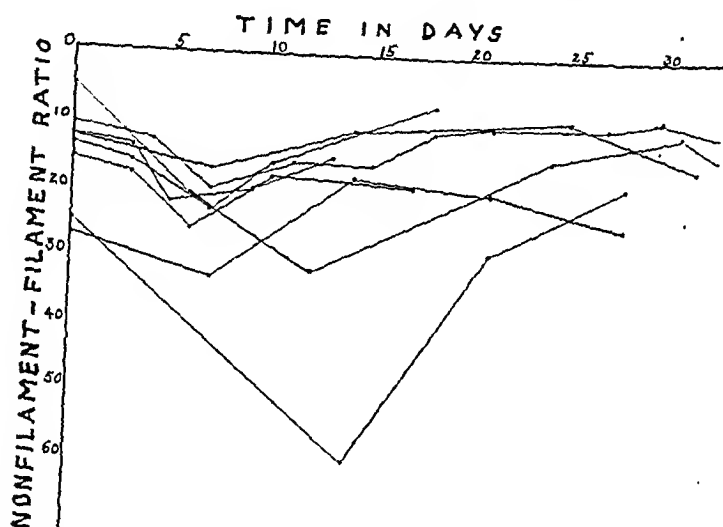


Fig. 6A.—Separate records of nonfilament-filament ratios used in computing Fig. 6.

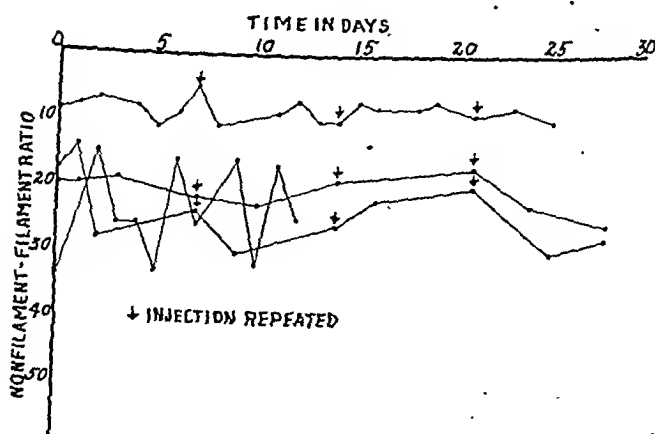


Fig. 7.—Nonfilament-filament ratios after injections of vaccine diluting fluid (0.5 per cent phenol) in 4 persons. Injections were made at the time of initial observation and were repeated as indicated.

e.g., during initial five-day periods (Figs. 1, 2, and 3), or in persons receiving injections either of diluting fluid (Fig. 7), or of vaccine too dilute to produce any subjective response (Fig. 8).

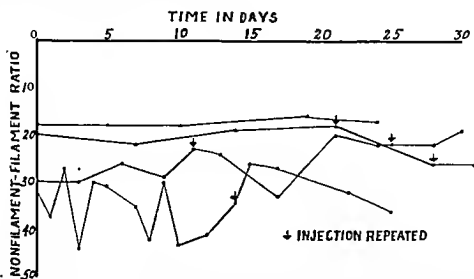


Fig. 8—Nonfilament-filament ratios of 4 persons in whom there was no evidence of subjective response following the injection of excessively diluted vaccine. (In Fig. 4C are shown the nonfilament-filament ratios of the same persons following the injection of stronger vaccines which produced type I responses.)

TABLE I

STATISTICAL ANALYSIS OF NONFILAMENT-FILAMENT RATIO CHANGES FOLLOWING THE INJECTION OF AUTOGENOUS VACCINES*

	NONFILAMENT-FILAMENT RATIO			
	NO	MEAN	S.D.	S.E.
Type I response				
Observations at time of injection	28	26.75	11.23	2.12
Observations during greatest improvement	28	11.57	4.60	0.87
Final observations	28	16.04	6.32	1.19
Type II response				
Observations at time of injection	12	18.42	4.31	1.30
Observations during most severe symptoms	12	30.08	12.29	3.72
Observations during greatest improvement	12	10.05	4.01	1.21
Final observations	12	14.25	2.97	0.895
Type III response				
Observations at time of injection	8	15.50	7.07	2.67
Observations during most severe symptoms	8	29.25	12.18	4.61
Observations during greatest improvement	8	13.00	4.34	1.64
Final observations	8	14.88	4.29	1.62

*No. indicates number of cases; S.D., standard deviation; S.E., standard error.

COMMENT

A statistical analysis of the nonfilament-filament ratio observations is presented in Table I. The differences between the means of the initial observations and the means of the observations at the point of most unfavorable symptoms were statistically significant (5.2 times the standard error in type II responses and 5.1 times the standard error in type III responses). Significant also were the differences between the means of the observations at the point of most unfavorable symptoms and at the point of greatest improvement (8.8 times the standard error in type II responses and 6.5 times the standard error in type III responses). The differences between the means of the initial observations and the means of the observations at the point of greatest improvement were statistically significant except in patients who experienced prolonged unfavorable

reactions (8.2 times the standard error in type I responses and 4.5 times the standard error in type II responses, but only 1.2 times the standard error in type III responses).

While in general the changes in the nonfilament-filament ratio paralleled the subjective changes, there were a few exceptions, probably because in these instances the symptoms might have been influenced by emotional, climatic, allergic, or other factors without corresponding changes in the leucocyte ratio.

SUMMARY

Nonfilament-filament counts were made at frequent intervals on persons showing definite subjective response to injections of antogenous vaccines. When there was an increase in symptoms, there was an increase in the nonfilament-filament ratio. When there was a lessening of symptoms, there was a lessening of the nonfilament-filament ratio. The variations in the nonfilament-filament ratio were statistically significant.

REFERENCES

- (a) Rawls, W. B., Gruskin, B. J., Ressa, A. A., and Jordon, M.: The Sedimentation Rate and Polymorphonuclear Count in Rheumatoid and Mixed Arthritis, *J. LAB. & CLIN. MED.* 19: 830, 1934. (b) Hench, P. S., Bauer, W., Fletcher, A. A., Christ, D., Hall, F., and White, P.: The Present Status of the Problem of "Rheumatism," *Ann. Int. Med.* 8: 1517, 1935. (c) Mullin, W. V., and Large, G. C.: The Filament-Nonfilament Count: Its Diagnostic and Prognostic Value, *J. A. M. A.* 97: 1133, 1931. (d) Eisenberg, A. A., and Nemens, H. S.: Value of the Schilling Hemogram in Infections, *Am. J. Surg.* 21: 56, 1933. (e) Crocker, W. J., and Valentine, E. H.: Hemography in Diagnosis, Prognosis and Treatment Based on Six Thousand Schilling Hemograms, *J. LAB. & CLIN. MED.* 20: 172, 1934.
- Stiles, M. H.: Sedimentation Rate and Nonfilament-Filament Ratio in Low Grade Chronic Illness. A Statistical Analysis of 292 Cases, *Arch. Int. Med.* 63: 664, 1939.
- Stiles, M. H.: Changes in the Sedimentation Rate and Nonfilament-Filament Ratio Following Clinical Improvement in Patients With Low-Grade Chronic Illness, *J. LAB. & CLIN. MED.* 26: 621, 1941.
- Stiles, M. H., and Chapman, G. H.: Frequency of Probable Pathogenic Streptococci and Staphylococci in Patients With Chronic Low Grade Illness, *Arch. Otolaryng.* 31: 458, 1940.
- (a) Chapman, G. H., Berens, C., Nilson, E. L., and Curcio, L. G.: The Differentiation of Pathogenic Staphylococci From Non-Pathogenic Types, *J. Bact.* 35: 311, 1938. (b) Chapman, G. H., Stiles, M. H., and Berens, C.: The Isolation and in Vitro Testing of Pathogenic Types of Non-exotoxigenic Streptococci, *Am. J. Clin. Path.* 9: 20, 1939. (c) Stiles, M. H., and Chapman, G. H.: Relationship Between Agglutinability and Certain in Vitro Tests of Staphylococci, Streptococci, and Colon Bacilli Isolated From Persons Suspected of Having Chronic Infection, *J. LAB. & CLIN. MED.* 24: 620, 1939. (d) Chapman, G. H., Berens, C., Lieb, C. W., Rawls, W. B., and Stiles, M. H.: Examination of Cultures From Persons Suspected of Having Chronic Infection, *Am. J. Clin. Path.* 9: 491, 1939.
- Stiles, M. H., Dirieckx, E. F., and Stiles, M. T.: The Normal Neutrophilic Leucocyte Count and Its Relation to the Schilling Count, *Am. J. Clin. Path.* 10: 100, 1940.
- Haden, R. L.: Qualitative Changes in Neutrophilic Leucocytes, *Am. J. Clin. Path.* 5: 354, 1935.

STUDIES ON ANTIPNEUMOCOCCIC RABBIT SERUM*

I. THE CORRELATION BETWEEN THE THERMAL REACTION TEST IN THE RABBIT AND THE INCIDENCE OF CLINICAL REACTIONS IN PNEUMONIA PATIENTS

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SABIN and Wallace¹ described a test for the presence of chill-producing substances in concentrated antipneumococcic horse serum in which dogs were injected intravenously with the serum. A rise in rectal temperature of 1.5° F. or more in about an hour indicated sufficient chill-producing material to cause reactions in patients treated with the serum. Barnes and Robinson,² using monkeys in a similar test, did not obtain as good agreement between their laboratory and clinical results.

Goodner, Horsfall, and Dubos³ have correlated thermal reactions produced in rabbits with incidence and severity of chill or febrile reactions in pneumonia patients when both were injected intravenously with the same antipneumococcic rabbit serum. They state that if the mean rise in temperature of a group of rabbits, each of which receives 2.0 ml. of serum, exceeds 1.2° F., the serum will produce chills in patients, and if the mean rabbit thermal reaction is over 2.0° F., the serum will produce such severe chills that it should not be used clinically. In their experience sera producing a thermal rise in rabbits of less than 1.2° F. gave no chills in patients. It was emphasized that the correlation was established only for sera prepared and processed by their procedure. Their sera were not concentrated but were treated with heat and kaolin.

It was desirable to know whether or not any such relationship existed in the case of concentrated antipneumococcic rabbit serum. During the development of refining and concentrating procedures for rabbit serum, more than 20 lots of concentrated serum were subjected to both clinical and rabbit tests, and a good degree of correlation was found.

Table I records the data. The rabbits employed for thermal tests were selected as follows: On the day of the test the rectal temperatures were taken. Animals with a normal temperature greater than 103.6° F. were not used. One milliliter of a freshly prepared pyrogen-free 0.9 per cent sodium chloride solution containing 0.2 per cent phenol and 0.01 per cent merthiolate was then injected intravenously, and the rectal temperature was taken at the end of one hour. If at this time the temperature had not varied more than 0.6° F. from its normal, the animal was then used for the thermal test of the serum.

*From the Biological Laboratories of E. R. Squibb & Sons, New Brunswick.
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Routinely 1.0 ml. of the concentrated serum was injected into an ear vein of a rabbit in one minute. (This dose is of the same order as that employed by Goodner, Horsfall, and Dubos with respect to the amount of protein administered.) The temperature was read one hour and two hours after the injection. The temperature difference at the one-hour interval was taken as the most significant, and has been used in the tabulations.

TABLE I

CLINICAL AND LABORATORY OBSERVATIONS ON CONCENTRATED ANTIPNEUMOCOCCIC RABBIT SERUM

CONCENTRATED SERUM			NUMBER OF PATIENTS		% INCIDENCE OF CHILL REACTIONS		TEMPERATURE	NUMBER OF RABBITS
TYPE	LOT	POTENCY (UNITS PER ML.)	TREATED	SHOW- ING CHILL REACTION	OBSERVED	CALCULATED*	RISE IN RABBITS (° F.) ± P.E.†	
I	1	10,000	65	2	3.1	3.6	0.20 ± 0.07	5
	2	10,000	9	3	33.3	28.1	0.87 ± 0.14	6
	3	12,000	19	9	47.4	32.5	0.94 ± 0.11	8
	4	10,200	4	1	25.0	6.6	0.36 ± 0.05	18
II	1	4,500	3	3	100.0	85.1	1.76 ± 0.10	24
	2	7,250	7	1	14.3	6.3	0.35 ± 0.09	13
	3	7,200	5	0	0.0	2.7	0.13 ± 0.06	14
III	1	2,500	14	11	78.6	82.9	1.71 ± 0.10	19
	2	9,450	11	2	18.2	25.1	0.82 ± 0.06	25
	3	16,300	9	1	11.1	11.2	0.52 ± 0.07	14
	4	14,500	3	1	33.3	8.1	0.42 ± 0.09	14
IV	1	8,400	6	2	33.3	55.2	1.26 ± 0.08	18
	2	10,950	3	1	33.3	3.4	0.19 ± 0.04	14
V	1	8,000	7	4	57.1	67.7	1.44 ± 0.13	8
	2	11,330	2	1	50.0	7.3	0.39 ± 0.06	20
VII	1	17,000	4	3	75.0	73.0	1.54 ± 0.13	8
	2	16,200	5	0	0.0	6.1	0.34 ± 0.05	14
	3	18,000	7	2	28.6	14.6	0.61 ± 0.06	22
VIII	1	7,870	5	1	20.0	31.8	0.93 ± 0.06	8
	2	16,700	3	0	0.0	5.7	0.32 ± 0.07	6
XIV	1	5,900‡	2	0	0.0	3.3	0.18 ± 0.02	14

*See text.

†Probable error = $0.6745 \sigma / \sqrt{n}$, where σ is the standard deviation and n is the number of rabbits used to test the lot of serum.

‡Calculated from antibody nitrogen.*

Five to 8 rabbits were used for each routine test. In many instances the test was carried out on the concentrates before and after sterile filtration. No significant differences were found in the two samples. All samples tested prior to sterile filtration were passed through a small Seitz E.K.S-3 asbestos pad to remove dust, lint, and other insoluble matter.

The potency of the antisera for types I, II, V, VII, and VIII pneumococci was determined by comparison in mouse protection tests with the standard sera distributed by the National Institute of Health. The sera for types III and IV were standardized in terms of Felton mouse protective units as determined in our laboratories, there being no official standard sera for these types. The

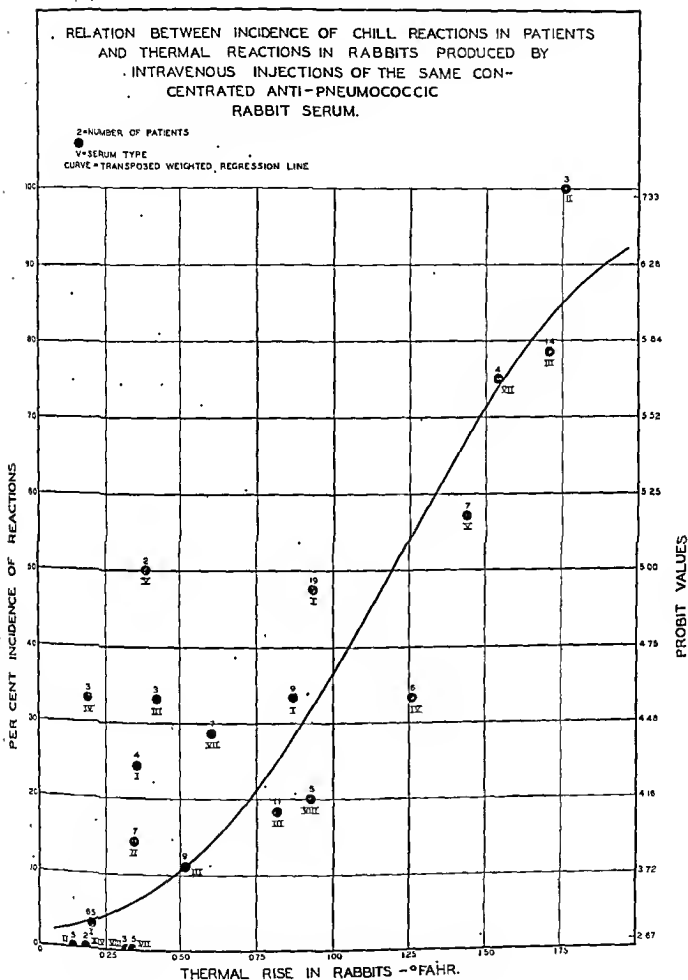


Fig. 1.

type XIV antiserum was evaluated by the quantitative precipitin reaction,⁴ 1 mg. of specifically precipitable antibody nitrogen representing 750 units, as recommended by the National Institute of Health.⁵

The clinical data are taken from reports received from 24 physicians located in various parts of the country.

Inspection of Table I reveals, for most lots, a definite relationship between the laboratory rabbit test and clinical observation. There is, of course, some dispersion which is particularly evident with lots for which only a few clinical reports were received.

The reports show that an average of 170,000 units of antibody was administered to each patient. In general, the chill or febrile reaction, when produced, occurred after the first large dose of about 50,000 units. Subsequent injections of the same serum were, for the most part, uneventful. Chills were usually severe if produced by a concentrated serum which caused a rise of 1.5° F. or more in rabbits. Potency or type of serum apparently had no significance as far as reactions were concerned.

It is not known what substance or substances in rabbit serum cause the thermal or chill reaction. In the statistical treatment of the data, it was assumed that the reactions were due to the same general type of pyrogenic substance which varied in amount from lot to lot. The thermal rise in rabbits was taken as a measure of the concentration or dose of pyrogenic material, and the incidence* of chill reactions in patients as an expression of the effect. This dose-effect relationship probably follows a typical sigmoid curve.

The data were plotted on arithmetic probability paper, and the percentage incidence of chill reactions was converted into probits.⁶ The weighted correlation coefficient† between the thermal rise in rabbits (in degrees Fahrenheit) and the probit values of percentage incidence of reactions in patients is 0.84, based on 193 patients and 292 rabbits treated with 21 lots of serum. In view of this high degree of correlation, a prediction equation was calculated. This equation is

$$I = 1.821T + 2.835,$$

where I is the probit value of the percentage incidence and T is the thermal rise in rabbits. Column 7 of Table I shows the value for incidence of chills as predicted by the above equation. The weighted average percentage deviation‡ of the observed from the calculated values is ± 7.0 per cent of the calculated values.

The sigmoid curve in Fig. 1 is a calculated weighted curve transposed from the regression line obtained on arithmetic probability paper, and is, therefore, a graphic expression of the prediction equation given above.

SUMMARY

A high degree of correlation (0.84) has been found between the temperature rise produced in rabbits and the incidence of chill reactions in patients with pneumonia following the administration of concentrated antipneumococcal rabbit serum.

An equation was developed permitting the prediction of incidence of chill reactions, the weighted average deviation of the calculated from the observed values being ± 7.0 per cent.

We wish to express our appreciation to Mr. H. A. Holaday and his associates of the Biological Assays Laboratories for carrying out the thermal tests in rabbits, and to Dr. G. F. Leonard and Dr. August Holm of the Bacteriological Laboratories for the mouse protection potency tests.

*Incidence is defined here as the percentage of patients treated with a given serum who developed a chill or chills. In general, the chill reaction was accompanied by a rise in temperature.

†Weights were assigned each observation in proportion to the number of patients treated with each lot of serum.

REFERENCES

1. Sabin, A. B., and Wallace, G. B.: On the Nature of the Chill-Producing Principle in Antipneumococcus Serum, *J. Exper. Med.* 53: 339, 1931.
2. Barnes, L. A., and Robinson, E. S.: Monkey Test for Chill-Producing Activity of Concentrated Antipneumococcus Serum, *Am. J. Pub. Health* 26: 51, 1936.
3. Goodner, K., Horsfall, F. L., Jr., and Dubos, R. J.: Type-Specific Antipneumococcus Rabbit Serum for Therapeutic Purposes: Production, Processing, and Standardization, *J. Immunol.* 33: 279, 1937.
4. Heidelberger, M., and Kendall, F. E.: The Precipitin Reaction Between Type III Pneumococcus Polysaccharide and Homologous Antibody. II. Conditions for Quantitative Precipitation of Antibody in Horse Sera, *J. Exper. Med.* 61: 559, 1935.
5. National Institute of Health: Memorandum to Licensed Manufacturers of Antipneumococcus Serum, Nov. 22, 1938.
6. Bliss, C. I.: The Calculation of the Dosage-Mortality Curve, *Ann. Appl. Biol.* 22: 134, 1935.

SPECIFIC CHEMOTHERAPY OF GIARDIA INFECTIONS*

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ALTHOUGH many widely different chemotherapeutic agents had been tried previously in the therapy of giardiasis, no effective drug was available until recent years for the permanent elimination of the giardias from man and animals. In 1937 Brumpt,¹ working with mice infected with *Giardia muris*, found that the administration by mouth of a 1 per cent solution of "quinacrine" (the French equivalent of atabrine) permanently cured 80 per cent of the infections. Later in the same year, Galli-Valerio² reported the successful elimination of *Giardia lamblia* from man by the administration of atabrine. A number of papers³ have since confirmed these first observations, and atabrine seems now fairly well established as an effective and safe drug for the treatment of giardiasis. The present communication offers further evidence upon the action of atabrine upon giardia infections of man, and of the albino rat and mouse.† The paper reports also that acriflavine, another acridine derivative, likewise is an effective drug in giardia infections of animals.

ATABRINE IN GIARDIA INFECTION OF MAN

Two cases of *Giardia lamblia* infection of man have been treated with atabrine. The first of these concerned an individual in whom the extraordinary persistence of the parasite has previously been reported by Miles and myself.⁴ Because this case responded so promptly to atabrine therapy, permanent cure being effected by a single course of treatment, the use of the drug in it will be described in some detail. The second human case, of which much less is known, will be only briefly mentioned.

Cure by Atabrine of a Long-Persisting Giardia Infection in Man.—The patient was a young woman of about 25 years of age, whose infection had been first

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†The atabrine used in the work was supplied by the Wmthrop Chemical Co., New York, N. Y.

diagnosed approximately six years earlier. At irregular intervals since the initial observation, the parasites or their cysts had been found in the patient's stools, search being made at least once each year and during some years frequently. An ineffectual attempt was made in the summer of 1936 by an attendant physician to eliminate the parasites by administering carbarsone. Cysts of the parasite were believed reduced in number thereafter for a time, but they never entirely disappeared. Soon after the cessation of treatment, they became as numerous as they had been originally.

Cysts of the parasite were observed in large number in January, 1940, one day prior to the institution of treatment with atabrine. Atabrine treatment was begun under the direction of Dr. C. W. Jungeblut of this department, two 0.1 Gm. tablets being given daily for five days. A stool specimen obtained two days after the last day of treatment was negative for cysts and trophozoites of *Giardia lamblia*, prolonged search being made of repeated samples of stool. Likewise, when stools obtained one, two, four, and six weeks later were examined, no cysts or trophozoites of *Giardia lamblia* could be found.

No significant by-effects of treating the patient with atabrine were noted. On the last day of treatment the patient felt listless and sneezed occasionally, but these symptoms were not certainly related to the use of the drug. No pigmentation of the skin was observed at any time. One day after the cessation of treatment, the stool was said by the patient to be somewhat watery and of a bright yellow hue, but subsequent stools were normal.

Cure by Atabrine of a Second Case of Human Giardiasis.—A second case of giardiasis in man was also treated successfully with atabrine, the drug being administered by Dr. H. M. Rose of this department. A stool examined just prior to the institution of treatment revealed many cysts of *Giardia lamblia*. Two 0.1 Gm. tablets of atabrine were given daily for five days. Stools obtained for three successive days after the last day of treatment were examined carefully, but neither cysts nor trophozoites of the parasite were seen. This patient has not since returned for observation.

ATABRINE IN GIARDIA INFECTION OF ALBINO RATS

Thirty rats naturally infected with *Giardia muris* were used to test the therapeutic action of atabrine upon giardiasis of rats, the infection being proved to exist in each rat by finding cysts of the parasite in a saline suspension of feces of the animal. All rats were small when used, weighing about 30 Gm. Atabrine in water solution was administered by mouth to 20 of the infected animals on two successive days, each dose being 10 mg. per 30 Gm. body weight. The drug was given by syringe through a blunted hypodermic needle (size 22; 1.5 inch length) lowered carefully into the pharynx or esophagus. The 10 remaining infected rats received no drug at any time, and were kept as controls of the infection in cages separate from the treated rats. The feces of the treated and control animals were examined daily for one week after the last dose of the drug. All the treated animals were free of the parasite, so far as fecal examination revealed by the first day after treatment, and remained so during the ensuing week (Table I). The 10 control rats retained their infections throughout this

period. Twelve of the treated rats were kept for three additional weeks, and their feces were examined at weekly intervals during this period. No animal revealed giardia cysts at any examination. At the end of this time these 12 rats were autopsied, and the small intestine was searched for trophozoites of *Giardia muris*. None was seen in any animal. It appears from this work that *Giardia muris* is promptly removed from the intestine of albino rats by the administration of 10 mg. of atabrine per 30 Gm. of rat body weight daily for two days, and that infections so treated do not relapse, at least during one month following treatment.

TABLE I

EFFECT OF ATABRINE AND OF ACRIFLAVINE UPON *GIARDIA* INFECTIONS IN DIFFERENT HOSTS

GIARDIA SPECIES	HOST SPECIES	DRUG USED	TOTAL DOSE OF DRUG GIVEN	NO. HOSTS TREATED	NO. HOSTS CURED	PERIOD OF OBSERVATION AFTER TREATMENT	UNTREATED CONTROLS	
							NO. USED	NO. POSITIVE FOR AT LEAST ONE WEEK
<i>Giardia lamblia</i>	Man	Atabrine	1 Gm.	2	2	3 days: 6 weeks	None	-
<i>Giardia muris</i>	Rat	Atabrine	20 mg.†	20	20	1 week to 1 month	10	10
<i>Giardia muris</i>	Mouse	Atabrine	20 mg.†	10	10	1 week	5	5
<i>Giardia muris</i>	Rat	Acridiflavine	20 mg.†	10	10	1 week	5	5

*One of the human cases was positive for over five years prior to treatment.

†Per 30 Gm. body weight.

It should be added that the rats tolerated the atabrine very well, and no animal was lost during the course of the experiment. The dose was, however, large in proportion to that given to the patients described earlier, and all the treated animals presented yellow pigmentation of the skin on the day after the second dose of drug was given. The pigment persisted for one week, and in some cases for longer periods. In several other rats given this dose of atabrine and autopsied one or two days thereafter, the small intestine was found deeply stained with the color of atabrine, particularly in those parts where *Giardia muris* is most commonly found.

ATABRINE IN *GIARDIA* INFECTION OF ALBINO MICE

Fifteen mice, all known from fecal pellet examination to harbor *Giardia muris*, were used to test the effect of atabrine upon mouse giardiasis. Ten animals were given 10 mg. of atabrine by mouth per 30 Gm. body weight on each of two successive days in the same manner as previously described for the infected rats. The remaining 5 mice were kept as controls for the natural persistence of the infection in the absence of treatment. The feces of all animals were examined daily for the presence of cysts of the parasite for one week, beginning one day after the last dose of drug. All treated mice were free of the infection, so far as fecal examination revealed, by the first day after treatment, and remained so throughout the period of observation (Table I). Autopsy at the end of this interval revealed no trophozoites of the parasite in the small intestine. The control mice showed cysts of the parasite in the feces at each of the daily observations until autopsy and then revealed the trophozoite stage in

the intestine. Apparently, cure of giardia infection by the administration of atabrine is about as successful in the mouse as it is in the rat or in man.

ACRIFLAVINE IN GIARDIA INFECTIONS IN ALBINO RATS

The remarkable effect obtained with atabrine upon the different giardiasis led to the trial of other acridine derivatives. One of these, acriflavine, a simpler substance than atabrine,* was also found to act powerfully upon giardiasis in the albino rat. This experiment was performed upon 15 albino rats, all proved by fecal examination to harbor *Giardia muris* prior to treatment. The same dose of acriflavine as that used with atabrine (10 mg. per 30 Gm. of body weight on two successive days) was given by mouth to 10 animals, the remaining 5 rats serving as controls. On the first day after treatment and throughout the week following, fecal examination revealed no cysts of *Giardia muris* in the treated animals, although the control rats continued to harbor the parasite throughout this period (Table I). At autopsy one week after treatment, no trophozoites of the parasite were found in the small intestine of the 10 treated animals. From this work it appears that acriflavine is an effective giardicide. It should be pointed out, however, that the drug in the dose used appeared to harm the rats, for, although none died, all lost weight.

TABLE II

EFFECT OF ATABRINE AND ACRIFLAVINE ON VARIOUS INTESTINAL PROTOZOA OF RATS

DRUG USED IN TREATMENT	NO. RATS IN GROUP	PERCENTAGE OF RATS HARBORING DESIGNATED PROTOZOA AT AUTOPSY					
		<i>Giardia muris</i>	<i>Hexamita muris</i>	<i>Chilomastix bettencourti</i>	<i>Trichomonas muris</i>	<i>Trichomonas parva</i>	<i>Endamoeba muris</i>
Atabrine	15	0	14	33	53	67	28
Acriflavine	11	0	9	72	45	72	27
None	23	21	30	65	60	73	56

Dose of drug: 10 mg. per 30 Gm. body weight daily for two days.

Autopsy was performed two days after last dose of drug.

EFFECT OF ATABRINE AND OF ACRIFLAVINE UPON CERTAIN INTESTINAL PROTOZOA OTHER THAN GIARDIA MURIS IN ALBINO RATS

An experiment was designed to test the effect of atabrine and of acriflavine upon certain intestinal protozoa other than *Giardia muris* found in albino rats. For this work, 49 rats of five different litters were used. The feces of these animals were not examined prior to treatment, but part of each litter was kept untreated as control. Fifteen rats were given atabrine and 11 were given acriflavine in the same dosage previously described (10 mg. per 30 Gm. body weight on two successive days); 23 rats received no drug treatment. Two days after the last dose of the drugs, all treated rats and controls were killed and their intestine searched for *Trichomonas muris*, *Trichomonas parva*, *Chilomastix bettencourti*, *Hexamita muris*, and *Endamoeba muris*, as well as for *Giardia muris*. In the case of none of the forms except *Giardia muris* was evidence forthcoming that either atabrine or acriflavine was regularly effective as a destructive agent, although in some instances the relative intensity of infection

*Atabrine: 2-methoxy 6-chloro 9-diethyl amino pentylamino acridine.
Acriflavine: 3, 6-diamino-acridinium-methyl chloride.

in the treated animals appeared somewhat less than in the control rats (see Table II). Of special interest was the persistence of *Hexamita muris* in the treated rats, since this parasite is closely related with *Giardia muris*. This experiment indicated that of the several species of protozoans found in the intestine of the albino rat atabrine and acriflavine exhibit a selective action upon giardia alone.

DISCUSSION

It is obvious from the data given in this paper, as well as from that of a number of other investigators who have previously studied the same problem, that atabrine is an effective drug for the cure of giardia infections of man and animals. Apparently, the giardias can now be placed with a slowly but constantly increasing list of parasites which are subject to control and elimination through the administration of specific chemical substances to the infected individual.

Atabrine appears to be a more favorable drug than acriflavine to use in the elimination of the giardias, judging from the experiments with *Giardia muris* of the rat, for rats treated with an equivalent dose of acriflavine lost weight and usually contracted respiratory infections, whereas those given atabrine showed none of these effects. Nevertheless, acriflavine was as effective as atabrine so far as the elimination of the parasite from the host was concerned.

The discovery of a drug which effectively cures giardia infections in man should render more soluble the old problem of the pathogenicity of *Giardia lamblia* in man. If the parasite is eliminated by atabrine treatment, then symptoms resulting directly from giardia infection should promptly disappear after treatment. On the other hand, if *Giardia lamblia* is not the cause of such symptoms, elimination of the parasite by atabrine cannot be expected to modify these symptoms. In one of the human cases reported in this paper, which was followed for a long period both before and after atabrine treatment, symptoms (epigastric pain, intestinal upset) were unchanged by the drug treatment. Evidently, the presence of the giardias was not responsible for the symptoms in this human case.

SUMMARY

Giardia parasites can be easily eliminated from man, and from albino rats and mice by the administration of atabrine. Apparently the infections are permanently cured by the drug, for relapses following treatment have not been observed in two human infections, one of which was carefully observed during six weeks thereafter, or in infections of laboratory animals which were followed for shorter periods. Reappearance of giardia parasites after treatment would seem, therefore, to occur only as the result of an exogenous reinfection contracted after the force of the drug were spent. The cure of the infection is rapid, no giardia cysts being seen even as early as the first day following the last dose of the drug in patients or in laboratory animals. Of especial interest is the fact that a single course of treatment sufficed to cure, presumably permanently, one of the human cases which had persisted previously for over five years.

Acriflavine, another acridine derivative, likewise will cause the disappearance of *Giardia muris* from the albino rat, although this drug seems less well tolerated by the animal than atabrine.

Neither atabrine nor acriflavine in the dose given to cure giardia infections is effective in eliminating infections with *Trichomonas muris*, *Trichomonas parva*, *Chilomastix bettencourti*, *Hexamita muris*, or *Endamoeba muris* from the albino rat.

I wish to thank Dr. C. W. Jungeblut and Dr. H. M. Rose, of this department, for their cooperation in this study.

REFERENCES

1. Brumpt, L.: Traitement experimental de la lambliaze, Compt. rend. Soc. de biol. 124: 1040, 1937.
2. Galli-Valerio, B.: La lambliaze et son traitement par l'atebrine, Schweiz. med. Wchnschr. 67: 1181, 1937.
3. Morrison, L. M., and Swalm, W. A.: A New Effective Parasiticide in Giardiasis, Am. J. Digest. Dis. 6: 325, 1939. Kouri, P. and others: Treatment of Lambliasis With Acridin Salts, Rev. de med. trop. y parasitol., bacteriol., clin. y lab. 4: 279, 1938. For a good review consult Clinical Excerpts, Vol. 13, No. 10, 1939, published by the Winthrop Chemical Co., New York, N. Y.
4. Miles, D. W., and Culbertson, J. T.: Persistence of Infection With *Giardia intestinalis* in Man, J. LAB. & CLIN. MED. 25: 286, 1939.

STUDIES OF THE EFFECT OF INTRAVENOUS ADMINISTRATION OF LIVER EXTRACT IN PATIENTS WITH SICKLE-CELL ANEMIA: AN UNUSUAL RESPONSE*

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A STUDY was undertaken to follow in detail the results of liver extract administered intravenously to patients with sickle-cell anemia.

MATERIAL AND METHODS

Four patients with sickle-cell anemia were studied. The blood was examined for ten to fifty days to establish the range of spontaneous variations. Twenty cubic centimeters of liver extract were administered intravenously, and daily counts were made of both white and red blood cells and reticulocytes for fifteen to forty days. In Case 4 (Fig. 1) additional counts were made at twenty minutes, three hours, five hours, and twelve hours following the administration of liver extract.

CASE 1.—T. P., 3 years old, was admitted with pains in the feet and ankles and with mild rhinopharyngitis. X-rays showed marked general cardiac enlargement and osteoporosis of the skull and long bones. Kahn and Wassermann tests were negative. Cholesterol was 110.3 mg. per 100 c.c. of blood, erythrocytes were 2,000,000 per c.mm. with 30 per cent reticulocytes, or 600,000 per c.mm., leucocytes were 10,000 per c.mm. with differential per cent as follows: lymphocytes 35.5, polymorphonuclear cells 54.5, mononuclear cells 6.0, eosinophiles 2.5, basophiles 0.5, and unidentified cells 1.0. Nucleated red blood cells and numerous sickle-cells were present. The blood count was not influenced by the liver extract.

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CASE 2.—W. M. J. was admitted with a leg ulcer of three years' duration and primary syphilis. Electrocardiogram showed slight myocardiac damage. Kahn test of blood was positive. Cholesterol was 139.13 mg., sugar 100 mg., nonprotein nitrogen 28.5 mg per 100 c.c. of blood. Erythrocytes were 1,900,000 per c.mm. with 22 per cent reticulocytes, or 418,000. Leucocytes were 25,000; differential per cent: lymphocytes 16.0, neutrophils 61.0, eosinophiles 15.0, monocytes 8.0. Volume index was 1.10. The patient was receiving weekly injections of neocarsphenamine while under observation. The blood and clinical course were not influenced by the administration of liver extract.

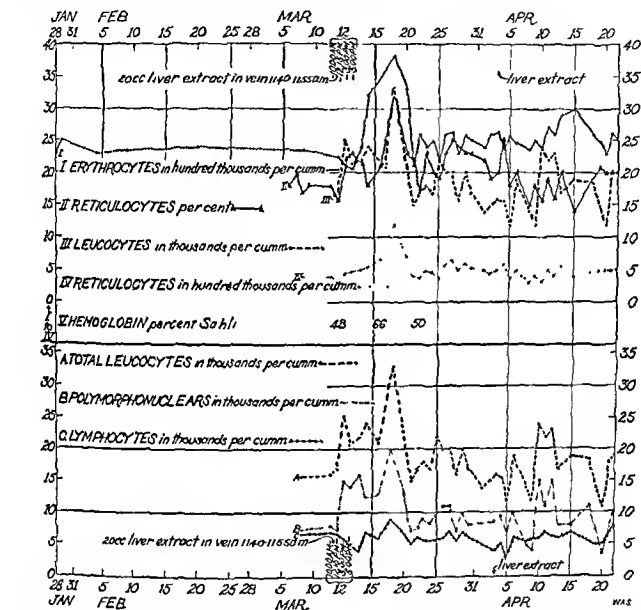


FIG. 1.—Chart showing leucocytes, erythrocytes, and reticulocytes per cubic millimeter and absolute number of the different types of white blood cells in relation to treatment (Case 4, R. R.).

CASE 3.—A. K., 38 years old, was admitted with joint pains and history of similar attacks in the past. He had received antisyphilitic treatment. On February 27 the icteric index was 7.7. Calcium was 9.4 mg., cholesterol 118.5 mg., uric acid 3.7 mg., organic phosphorus 3.6 mg., sugar 91.0 mg., nonprotein nitrogen 27.5 mg. per 100 c.c. of blood. On March 2 bilirubin was 4.16 mg. per 100 c.c. of blood; icteric index was 8.0. Erythrocytes 4,000,000 with reticulocytes 16 per cent, or 640,000 per c.mm. Nucleated erythrocytes were 5,140 per c.mm. Leucocytes were 18,000; differential per cent: lymphocytes 31.0, polymorphonuclear cells 54.0, eosinophiles 3.0, basophiles 2.0, monocytes 10.0. The administration of liver extract did not influence the blood picture.

CASE 4.—R. R., aged 14 years, was admitted with a leg ulcer. He had been admitted 12 years previously during an abdominal crisis. On previous admission (Sydenstricker) he had 1,541,000 erythrocytes with 34 per cent reticulocytes, or 524,000 per c.mm. Leucocytes were 20,000. During present admission his count was erythrocytes 2,300,000 with 18 per cent reticulocytes, or 414,000 per c.mm. Leucocytes were 16,000; differential per

lymphocytes 44.0, neutrophils 48.0, eosinophils 3.0, basophils 1.0, monocytes 4.0. Numerous sickle cells and few nucleated erythrocytes were found. Volume index was 110-118. Three hours after liver extract was administered, leucocytes showed an increase and maintained this rise for nine days. The erythrocytes rose on the third day, and the reticulocytes showed a definite rise on the fifth day. The rise was progressive for all three elements, and on the seventh day they all reached the peak: erythrocytes 3,800,000 with 32 per cent reticulocytes, or 1,200,000 per c.mm. and leucocytes 33,000. On the ninth day the blood count returned to the level noted before treatment. The rise of leucocytes was due entirely to the increase of mature polymorphonuclear cells. The proportion of sickle cells was practically unaltered. The hemoglobin rose, but not in proportion to the rise of the erythrocytes. On the day of administration hemoglobin (Sahli) was 48.0 per cent with 2,300,000 red blood cells; on the fifth day hemoglobin was 66 per cent with 3,555,000 erythrocytes. It returned to the previous level simultaneously with the erythrocytes. Twenty-three days later the same dose of liver was repeated, and on the eighth day a moderate rise of polymorphonuclear cells was observed up to 24,000 per c.mm.; this rise lasted three days. On the eleventh day the erythrocytes rose for two days, but there was no rise in reticulocytes (Fig. 1).

DISCUSSION

Sydenstricker² pointed out that the treatment of sickle-cell anemia is most unsatisfactory. Diggs³ published charts illustrating the negative results in the treatment of sickle-cell anemia by liver extracts.

Following intravenous injection of liver extract, Meyer, Middleton, and Theulis⁴ found depression of leucocytes within twenty minutes in normal persons and in patients with blood dyscrasias; this depression was followed by a leucocytosis which reached the maximum three hours later. Miller and Rhodes,⁵ in an anemic woman with splenomegaly, found increase of leucocytes in twenty minutes, as well as an increase in the other formed elements of blood, with return to normal in twenty-four hours; the rise of blood count was simultaneous with contraction of the spleen.

The case of R. R. seems to be unique in its response to liver extract. The rise of erythrocytes before the increase in reticulocytes is different from that observed in pernicious anemia. The volume index was slightly higher than normal, but this is frequent in cases of sickle-cell anemia when the reticulocytes are high. The spleen was not palpable and was thought to be greatly diminished, as in sickle-cell anemia. No abnormal blood pressure changes were noted on the day of administration or later. The independent appearance of the rise of leucocytes and erythrocytes, and the duration of this rise, exclude the possibility of its being the result of any kind of redistribution. I feel compelled to consider it the result of action on the hematopoietic system, although the previous administration of liver by mouth and of liver extract intramuscularly was without effect.

SUMMARY

1. The results of the intravenous administration of liver extract to 4 patients with sickle-cell anemia are reported, with 3 patients showing no response and one patient showing unusual response of the hematopoietic system.
2. This response consists in the increase of polymorphonuclear leucocytes, erythrocytes, and reticulocytes on the first, third, and fifth days, respectively, with simultaneous return to previous level on the tenth day.
3. This is considered as the result of action of the extract on the hematopoietic tissue.

REFERENCES

1. Sydenstricker, V. P., Mulherin, W. A., and Houseal, R. W.: Sickie-Cell Anemia: Report of 2 Cases in Children, With Necropsy in 1 Case, *Am. J. Dis. Child.* 26: 132, 1923.
2. Sydenstricker, V. P.: Sickie-Cell Anemia, *M. Clin. North America* 12: 1451, 1929.
3. Diggs, L. W.: Negative Results in the Treatment of Sickie-Cell Anemia, *Am. J. M. Sc.* 187: 521, 1934.
4. Meyer, O. O., Middleton, W. S., and Theulis, E. M.: Leucocytosis After Parenteral Injection of Liver Extract, *Am. J. M. Sc.* 188: 49, 1934.
5. Miller, D. K., and Rhodes, C. P.: The Effect of Splenic Contraction on the Formed Elements of the Blood in a Case of Anemia and Splenomegaly, *J. Clin. Investigation* 12: 1009, 1933.

STUDIES IN BLOOD PRESERVATION*

FATE OF CELLULAR ELEMENTS AND PROTHROMBIN IN CITRATED BLOOD

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IN A previous article² we reported the changes observed in blood preserved with heparin. The results presented in this study are typical of a large number of observations on citrated blood.

METHOD

Five cubic centimeters of freely flowing venous blood were collected in each of thirty-five sterile flat-bottomed tubes containing 0.5 c.c. of 3.5 per cent sodium citrate solution as the anticoagulant. The blood was kept in a refrigerator at a temperature of from 4° to 6° C. throughout the period of the experiment.

Each day one tube was taken from the refrigerator and the following determinations were made: red blood cell count, hemoglobin, white blood cell count, differential white blood cell count, platelet count, and fragility test.

The plasma clotting time was done on two samples of 50 c.c. of blood by the method of Quick,^{7,8} who postulates that the rate of coagulation is a function of the concentration of prothrombin, and the production of thrombin in oxalated plasma is proportional to the concentration of prothrombin if an excess of thromboplastin is present and an optimal amount of calcium is added. The plasma was recalcified with calcium chloride at a constant temperature of 40° C. in the presence of human brain tissue emulsion to supply the thromboplastin substance, the end point being recorded by the shift on a photoelectric cell galvanometer.

*From the Surgical Pathology Laboratory of the College of Physicians and Surgeons, Columbia University, New York.

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RESULTS

Donor, W. T. S.

COMPARATIVE VALUES OF BLOOD IN HEPARIN AND IN SODIUM CITRATE

	HEPARIN	SODIUM CITRATE 0.35 PER CENT
Hematoerit (3)	48.5	42.3 per cent cells
Plasma specific gravity (3)	1.0249	1.0238
Plasma proteins (3)	6.12	5.75 Gm. per cent
Whole blood potassium (10)	192.0	183.0 mg. per cent
Plasma potassium (10)	17.5	17.2 mg. per cent
Cell potassium (calculated) (10)	377.0	409.0 mg. per cent

VALUES ON INITIAL SAMPLE CORRECTED FOR DILUTION IN CITRATE

Red blood cell count	5,100,000.0
Hemoglobin	15.6 Gm. per cent
White blood cell count	7,700.0
Differential white blood cell count	
Polymorphonuclear leucocytes	61.0 per cent
Lymphocytes	29.0 per cent
Monocytes	7.0 per cent
Eosinophilic leucocytes	2.5 per cent
Basophilic leucocytes	0.5 per cent
Platelets	206,800.0

The results of the counts and hemoglobin determinations are graphically represented in Figs. 1, 2, and 3.

Fragility of Erythrocytes.—The end points were poor throughout these studies. An actual curve of fragility could not be constructed on a daily basis. As late as the fifteenth day cells could be suspended in 0.45 per cent sodium ehloride without complete hemolysis. Even on the thirtieth day cells carefully handled did not lake completely in 0.52 per cent sodium ehloride.

Spontaneous hemolysis was first noted on the seventeenth day. Slight shaking in physiologic saline after the tenth day caused hemolysis. The cells on the tenth day are slightly less resistant than those on the first, and those stored for thirty days are definitely more fragile than those stored for ten days.

Prothrombin.—In the first series plasma clotting times were run for a period of fifty-two consecutive hours at one-hour (toward the end, two-hour) intervals. This was done with the aid of Dr. Kenneth Olsen. There was a rapid rise in clotting times in the first fifteen hours; at the end of this period the prothrombin content had been reduced to ineffectual levels. The curve was similar to that later published by Rhoads and Panzer⁹ and seemed to indicate that bloods stored for periods longer than a day would be ineffectual in treating hemorrhage which resulted from a deficiency of prothrombin.

Eight-day-old blood, however, was given a jaundiced patient with a hemorrhagic tendency, and bleeding stopped. This was reported to Dr. Olsen who made up a new extract of rabbit brain and repeated these tests on blood supplied to him from the blood bank. To his surprise this time there was no sudden loss of prothrombin concentration.

Through the courtesy of Dr. Grant Sanger we have been able to obtain the observations tabulated in Table I on separate bloods, each time being very careful to use fresh brain extract.

CELLULAR CHANGES IN CITRATED BLOOD

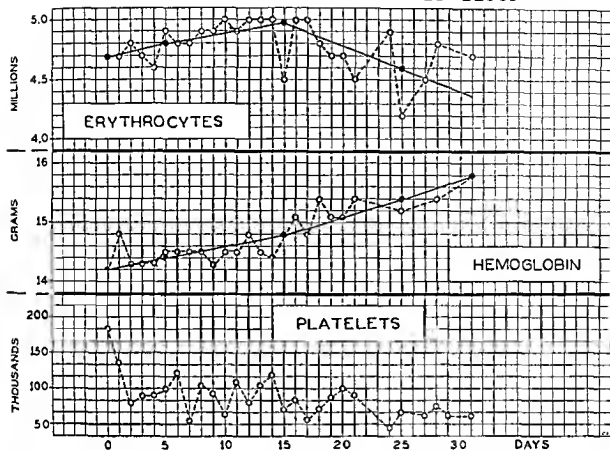


Fig. 1.—Red blood cell counts, corrected for dilution, varied between 5.5 and 4.6, the mean being 5.1 millions. Here there is an actual loss of 1,000,000 to 1,500,000 cells at the end of thirty days. Hemoglobin values varied between 13.6 and 16.3 Gm. per cent. The gradual rise is attributed to evaporation. Platelets fell from 206,800 to 87,800 in forty-eight hours, then remained constant for about fifteen days, after which time counts were difficult.

CELLULAR CHANGES IN CITRATED BLOOD

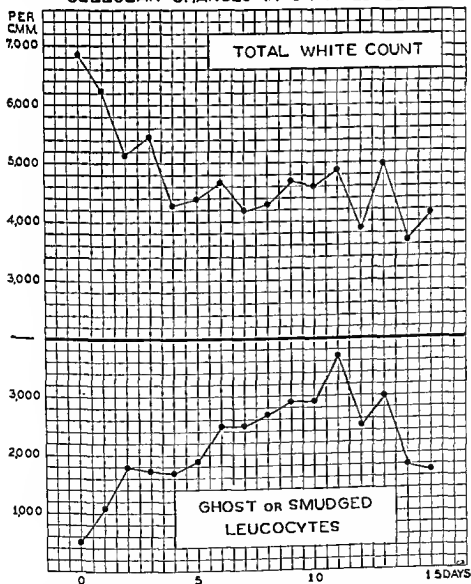


Fig. 2.—The total white blood cell count as observed in the chamber fell 27 per cent in the first five days. Careful differentiation of cells in the chamber and observation on a slide showed that 75 per cent of the total had no nuclei or were so fragile that they broke and left only a smudge by the twelfth day.

DISCUSSION

Red Blood Cells.—The maintenance of total erythrocyte counts at an approximately constant level in heparinized blood² and the moderate destruction of erythrocytes in citrated blood after the fifteenth day of storage suggest that cell destruction plays, at most, only a small part in the steady increase of the potassium content of the plasma,¹¹ and insures the recipient of receiving a large percentage of functioning cells after periods of storage of at least a month.

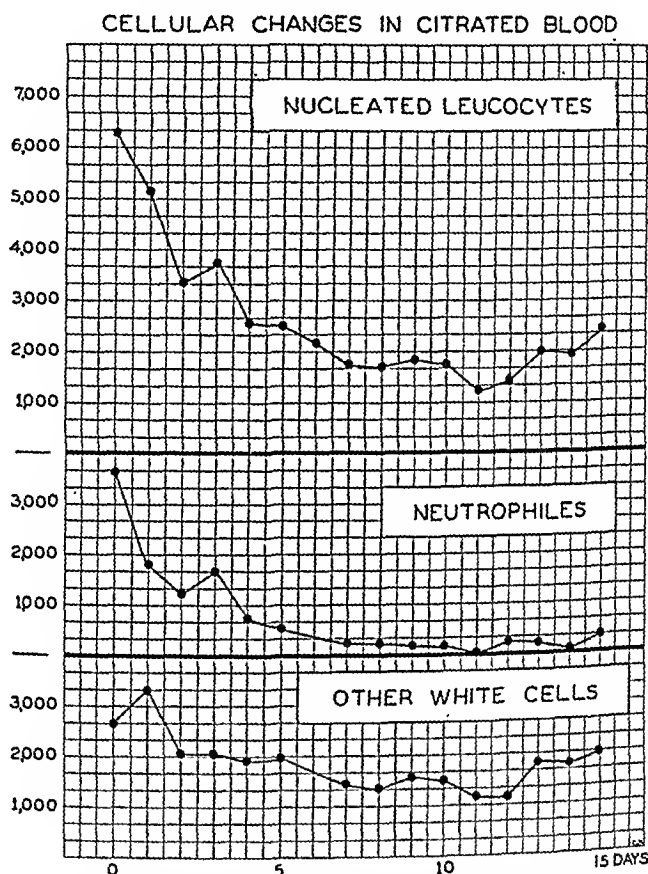


Fig. 3.—The nucleated leucocytes, presumably the only ones capable of function, decreased nearly 50 per cent in the first three days. The polymorphonuclear leucocytes diminished 50 per cent in twenty-four hours, accounting almost completely for the drop in total count. By the sixth day it was difficult to be sure that any remained. Eosinophiles were well preserved. Lymphocytes remained almost constant. Monocytes were difficult to differentiate.

Hemoglobin.—The hemoglobin content of the whole blood remains constant, though at the end of thirty days 20 per cent of it may be found in the plasma.

White Blood Cells.—The polymorphonuclear leucocytes may show swelling, hazy cytoplasm, and poorly staining nuclear granules as early as twenty-four hours after storage. Disintegration is extremely rapid and may be responsible for the steep rise of the potassium curve in the first week;¹¹ likewise it may play a part in decreasing bactericidal properties.^{4, 5}

The lymphocytes are more resistant. At the end of thirty days they are easy to recognize when seen.

The eosinophiles are most resistant, the eosinophilic granules remaining particularly bright even when the nuclear material has faded or broken up.

Platelets.—Platelets disintegrate more rapidly in heparinized blood² than in citrated blood. In both bloods they reach their low point in about three to five days. Platelets remaining stay fairly fixed until clumping makes counting difficult about the fifteenth day.

TABLE I
PROTHROMBIN CONCENTRATIONS IN PLASMA OF STORED BLOOD

DATE BLOOD STORED	DATE TEST MADE	AGE IN DAYS	PROTHROMBIN CONCENTRATION (PER CENT)
10/27/39	2/21/40	117	47
10/27/39	2/21/40	117	38
11/20/39	2/21/40	94	41
12/15/39	2/21/40	69	49
1/31/40	2/21/40	21	52
2/28/40	3/12/40	13	100
3/ 1/40	3/12/40	11	100
2/16/40	2/26/40	10	68
2/23/40	3/ 1/40	8	84
2/23/40	3/ 1/40	8	84
2/26/40	3/ 5/40	8	60
3/ 4/40	3/12/40	8	100
3/ 4/40	3/12/40	8	80
3/ 4/40	3/12/40	8	100
2/19/40	2/26/40	7	100
2/19/40	2/26/40	7	76
2/23/40	2/29/40	6	100
2/23/40	2/29/40	6	100
2/16/40	2/21/40	5	74

Prothrombin.—The results using the Quick method of determining the prothrombin concentration yield values similar to those reported by Lord and Pastore⁶ using the Brinkhous, Smith, and Warner method.¹ This suggests that the use of brain extract, which has been kept too long, may account for the discrepancy between reported findings. Dr. Olsen deserves credit for making this very essential observation.

SUMMARY

1. In citrated blood there is some loss in the number of red blood cells beginning about the fifteenth day and amounting to from 1,000,000 to 1,500,000 cells by the end of the month.
2. The hemoglobin content remains constant in the total sample, although 15 to 25 per cent may diffuse out of the cells into the plasma in one month.
3. The polymorphonuclear leucocytes are diminished to 50 per cent in forty-eight hours and are amorphous masses in fifteen days.
4. The lymphocytes and eosinophiles do not disintegrate so rapidly; the latter are particularly well preserved. The monocytes are difficult to trace.
5. The platelets fall rapidly to a level ranging from 50,000 to 80,000 and remain at this level for about fifteen days, at which time counts become difficult to make.
6. The fragility of red blood cells slowly increases with increasing age; exact curves are difficult to establish.
7. The prothrombin level is maintained above 40 per cent of normal concentration for a period of at least four months. The use of old brain extract

will cause clotting times which are too rapid, thereby giving a false picture of the true degree of efficacy of preserved blood in the therapy of hemorrhagic diseases associated with low prothrombin concentrations.

We are indebted to Miss Hildegard Menzel for aid in the prothrombin determinations and to Mr. Josiah Lasell for many hematologic studies.

REFERENCES

1. Brinkhous, K. M., Smith, H. P., and Warner, E. D.: Plasma Prothrombin Level in Normal Infancy and in Hemorrhagic Disease of the Newborn, *Am. J. M. Sc.* 193: 475, 1937.
2. Drew, C. R., Edsall, K., and Scudder, J.: Studies in Blood Preservation. Fate of Cellular Elements in Relation to Potassium Diffusion, *J. LAB. & CLIN. MED.* 25: 240, 1939.
3. Drew, C. R., Scudder, J., and Papps, J.: Controlled Fluid Therapy With Hematocrit, Specific Gravity, and Plasma Protein Determinations, *Surg., Gynec. & Obst.* 70: 859, 1940.
4. Jordá, F. D., and Llorach, A. B.: Contribución al estudio del metabolismo in vitro de la sangre citratada-conservada. La destrucción del complemento, *Rev. sanidad de Guerra* 1: 344, 1937.
5. Kolmer, J. A.: Preserved Citrated Blood "Banks" in Relation to Transfusion in the Treatment of Disease With Special Reference to the Immunologic Aspects, *Am. J. M. Sc.* 197: 442, 1939.
6. Lord, J. W., Jr., and Pastore, J. R.: Plasma Prothrombin Content of Bank Blood, *J. A. M. A.* 113: 2231, 1939.
7. Quick, A. J., and Leu, M.: Quantitative Determination of Prothrombin, *J. Biol. Chem., Proc. Am. Soc. Biol. Chem.* 119: lxxxi, 1937.
8. Quick, A. J., Stanley-Brown, M., and Baneroff, F. W.: A Study of the Coagulation Defect in Hemophilia and in Jaundice, *Am. J. M. Sc.* 190: 501, 1935.
9. Rhoads, J. E., and Panzer, L. M.: The Prothrombin Time of "Bank Blood," *J. A. M. A.* 112: 309, 1939.
10. Scudder, J.: Shock: Blood Studies as a Guide to Therapy, Philadelphia, 1940, J. B. Lippincott Co.
11. Scudder, J., Drew, C. R., Coreoran, D. R., and Bull, D. C.: Studies in Blood Preservation, *J. A. M. A.* 112: 2263, 1939.

EFFECT OF VITAMIN C ON WORKERS EXPOSED TO LEAD DUST

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THE present study deals with a group of 303 regular employees, 80 per cent of whom have been in their present place of occupation for five years, and the balance for a longer period. These men are engaged in various types of work in the assembly of passenger automobiles.

The lead exposure in this work of assembly arises from the practice of covering body seams with solder (60 per cent lead), and grinding off the excess metal to form a flush surface. There is thus thrown into the air a large quantity of lead dust which is of variable particle size, the majority of particles being too large to be inhaled.

In a large series of air samples it was determined by standard methods that there was a variable concentration along the assembly line of inhalable lead dust (10 microns or less in size). This concentration varied from 3 to 15 mg. per 10 cubic meters of air.

The concentrations of dust and the particle size findings coincide with the report of Humberdine¹ on similar lead hazards in European automobile assembly plants.

Provision for ventilation consists of overhead and floor level exhaust ducts; and grinders and metal finishers are furnished with daily inspected respirators. Floors are kept clean by wet sweeping.

Since early in 1935 all men who were even remotely in contact with lead dust have been examined physically, and a basophilic aggregation count was made (McCord)² once a month, or oftener if complaints arose.

During the period in 1935 in which a wave of hysteria concerning lead poisoning was at its height, there were 340 definite complaints that ranged from abdominal cramps, nausea, and vomiting, to alleged weakness of the extensor muscles of the wrists and forearms. The majority of these illnesses were patently of psychologic origin, but there were 13 men in this group who were hospitalized for further observation and possible treatment for lead intoxication.

Urinalysis for lead, both before and after deleading treatment, failed to show an abnormal excretion of lead in any one of these 13 men; and the basophilic aggregation count, with the exception of three of the group, did not bear out a diagnosis of lead intoxication.

The three men who had a high basophilic aggregation were variously found to have a severe pyorrhea alveolaris and chronic cryptic tonsillitis, chronic appendicitis, and peptic ulcer. When treatment appropriate to these conditions was instituted, the blood picture returned to normal, and the lead poisoning disappeared.

At this time a routine form of medication for those exposed to lead dust was initiated. This treatment consisted of the administration once a week—Monday—of 1 ounce of a saturated solution of magnesium sulfate, to which was added 15 grains of citric acid and sufficient tincture of eudbear to color the mixture; this was given to the men when they obtained their tools, thus insuring that practically all the men would receive medication before starting work. The results of this treatment, judged by the basophilic aggregation count and lack of serious complaints, were good, but were met with strenuous objection from the men.

Beginning in June, 1939, the foregoing medication was discontinued, and in its place each man was given two pieces of gum containing a total of 50 mg. of ascorbic acid. These were distributed about one-half hour after the noon lunch period. The men made no objection to taking the gum, and considered it a treat. Physical examinations and basophilic aggregation counts were continued as previously.

After a short period there was a noticeable decrease in muscular fatigue. This was evident by an increase in efficiency and a feeling of well-being at the end of the work period. The men seemed more cheerful and much less inclined to moroseness.

During the five months from June to December, 1939, the basophilic aggregation count showed a slow but progressive decline. At this time further laboratory studies were undertaken as follows: (a) urinalysis for lead, (b) basophilic aggregation count, and (c) a stipple cell count. These determinations were made each week for seven weeks, covering a period of thirty-five working days of eight hours each.

During this test period production was at its peak, humidity was high, and there was a slow circulation of air due to the low temperature of the outside air.

Urine was collected at different periods of the working day to obtain a fair average of lead excretion. A total of 1,996 analyses for lead were made, or an average of 6.5 analyses per man.

The results of the lead urinalysis are shown in Table I.

TABLE I

NO. OF MEN	PER CENT	AVERAGE OF URINARY LEAD EXCRETION PER LITER (γ)
99	32.6	39.5
96	31.6	61.5
48	15.8	86.2
24	7.9	110.4
14	4.6	134.5
10	3.3	160.9
5	1.6	189.6
3	0.9	219.3
1	0.3	265.0
2	0.6	405.0
1	0.3	541.0

Those below 100 γ per liter, 243 men, or 80.2 per cent, averaged 62.4 γ per liter.

Those above 100 γ per liter, 60 men, or 19.8 per cent, averaged 157 γ per liter.

The average over-all excretion of 303 men equaled 77 γ per liter.

In this series was one man who excreted an average of 541 γ per liter. Further questioning revealed that he had had acute lead intoxication several years previously. He had not worked with lead for eight years, and was at least two city blocks away from any lead; therefore, his exposure has been nil. Under the influence of vitamin C, we are evidently releasing the fixed lead from his bones and he is excreting it without showing any clinical symptoms of lead intoxication.

The workers who showed an average of more than 100 γ per liter of urine—60 men—were studied further. The urine and blood counts were continued at fortnightly intervals. At the end of April, 1940, the lead excreted in the urine has shown no decline, and the basophilic aggregation and stipple cell counts have remained within the normal range.

DISCUSSION

From the foregoing it is apparent that this study confirms the contention of Holmes³ that vitamin C (ascorbic acid) has a detoxifying action on lead in the human body. However, we cannot agree that the urinary lead excretion is decreased by the administration of vitamin C—since our findings show a continued high level of excretion over an observed period of five months in those workers continuously exposed to a high concentration of lead dust.

It is difficult to reconcile our findings with those of Shiels,⁴ and Blumberg and Scott⁵; i.e., that there are definite levels of concentrations for lead, both in urine and blood, above which levels, one finds lead poisoning. Judged by the criteria of blood cell counts, physical condition, and lack of symptoms usually associated with chronic lead absorption, the subjects of this study may be said to show no evidence of lead intoxication whatever. Fairhall⁶ has stated that a high urinary excretion of lead is not of itself evidence of lead intoxication, but rather of lead absorption only.

We have found no evidence that the storage of lead is promoted by vitamin C. Rather the reverse is true, as shown by the workman with the highest level of excretion in the group. As stated, this man has been previously exposed and has been treated for lead intoxication.

Further investigation in the deleading properties of vitamin C are being undertaken.

It seems possible that the effect of lead on the human economy may be only indirectly toxic, the absorption of lead producing an avitaminosis C. This is the condition of vague symptoms known as chronic lead poisoning, but is in reality chronic subclinical scurvy.

CONCLUSIONS

1. The routine administration of 50 mg. of ascorbic acid daily appears to protect workers exposed to lead dust against the usual effects of chronic lead absorption.

2. The rate of urinary excretion of lead in workers continually exposed to lead dust is not decreased by the routine daily administration of 50 mg. of ascorbic acid.

I wish to express my appreciation to Dr. Milton H. Kronenburg, Chief of Division, and K. M. Morse, Engineer, of the Illinois Industrial Hygiene Bureau, for the air-borne dust determinations; and to Doctors E. K. Nielsen and H. A. Cranston for the determinations of urinary lead.

REFERENCES

1. Humperdinck, C.: Schwankungen des Hämoglobins und der Zahl der weissen Blutkörperchen bei mehrfacher, hintereinander erfolgender Blutentnahme aus einer Schnittwunde am Ohrfläppchen, *Arch. f. Gewerbepath. u. Gewerbehyg.* 8: 532, 1938.
2. McCord, C. P., and Johnson, J.: The Basophilic Aggregation Test, *Am. J. Pub. Health* 25: 1089, 1935.
3. Holmes, H. N., Campbell, K., and Amberg, E. J.: Effect of Vitamin C on Lead Poisoning, *J. LAB. & CLIN. MED.* 24: 1119, 1939.
4. Shiels, D. O.: Concentration of Lead in Urine of Workers at Mount Isa Mines Limited, Queensland, With Special Reference to Its Value in Diagnosis of Lead Poisoning, *M. J. Australia* 1: 559, 1936.
5. Blumberg, H., and Scott, T. F. M.: Quantitative Spectrographic Estimation of Blood Lead and Its Value in Diagnosis of Lead Poisoning, *Bull. Johns Hopkins Hosp.* 56: 276, 1935.
6. Fairhall, L. T.: Significance of Excretions of Lead in Urine, *U. S. Pub. Health Rep.* 54: 2016, 1939.

7255 YALE AVENUE

CLINICAL CHEMISTRY

A COMPARISON OF THE HEMOGLOBIN RESPONSE TO VARYING DOSAGES OF IRON*

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ALTHOUGH the efficacy of simple iron salts in the treatment of iron deficiency hypochromic anemias has been adequately demonstrated, there is still a question as to the relative effectiveness of various sized dosages. The usually recommended doses are large and the present consensus is that a massive dose of iron is the advisable method of therapy. Balance studies have shown, however, that sufficient iron is retained from the administration of a smaller amount of the drug, so that adequate quantities are available for hemoglobin formation as well as for replenishment of the depleted reserves.^{1, 2} From those data it would seem that doses smaller than those usually recommended should produce a satisfactory increase in hemoglobin. The danger of large amounts of iron has been suggested by Elvehjem³ and others,⁴ and although we were unable to demonstrate that iron significantly affected the metabolism of calcium or phosphorus in the adult,⁵ the large doses may not be without danger. It was also shown in balance studies that large amounts of iron were retained by the body over long periods of time,¹ and although only one instance of a severe reaction to orally administered iron has been recorded,⁶ it does not seem wise to overburden the body with an excess such as must occur with the commonly employed doses when administered over a long period.

It has been demonstrated that large doses of iron produce a more rapid response in hemoglobin formation than do smaller amounts⁷⁻¹⁰ and that ferrous salts are more effective than ferric salts when compared on a basis of their metallic iron content.^{11, 12} Moore¹³ has shown that the serum iron absorption curves are higher following the ingestion of ferrous than of ferric salts. In order to compare the effectiveness of varying sized doses of iron and of various iron salts, we have administered these preparations to groups of patients with mild but comparable degrees of anemia under identical conditions.

From balance studies with iron and ammonium citrates the amount of iron excreted, retained, and utilized from a given dose was known,^{1, 2} so that this drug was selected for the first of the clinical trials. All subjects selected were healthy young adults, with no illness except the mild anemia which was detected on routine examination, and none was studied who had had a recent infection, hemorrhage, or other disability which might affect the blood hemoglobin. Hemoglobin determinations were done by the Newcomer method,¹⁴ hematocrits were

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determined by the Van Allen method,¹⁵ and erythrocyte counts were done on instruments certified by the United States Bureau of Standards. In the accompanying charts only the hemoglobin changes are recorded, although hematocrit readings and erythrocyte counts were done at the time of each hemoglobin determination.

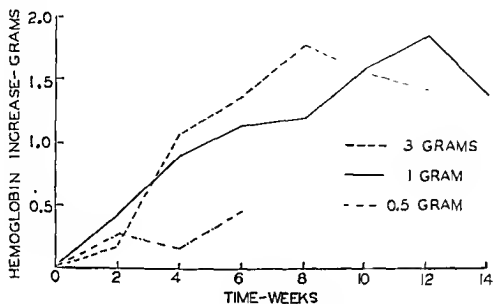


Chart 1.—Iron and ammonium citrates.

To one group of 18 persons was given 0.5 Gm. (7½ grains) of iron and ammonium citrates per day. The average hemoglobin level for this group was 9.73 Gm. per 100 c.c. The average hemoglobin gain for the entire group in a period of six weeks is shown in Chart 1, and it will be noted that although there is an increase in hemoglobin, the gain is too slow to be considered a satisfactory response. During a subsequent six-week period 1.5 mg. of copper sulfate were added to the same dose of iron and the hemoglobin gain was no more rapid than without the additional copper.¹⁶ This amount of iron and ammonium citrates yields approximately 85 mg. of elemental iron per day, but from the results recorded here it is obvious that this amount of iron in the form of iron and ammonium citrates is inadequate to produce a satisfactory clinical response.

To a similar group of 15 persons, whose initial hemoglobin averaged 11.53 Gm., was given 1 Gm. (15 grains) of iron and ammonium citrates per day. This yields approximately 170 mg. of elemental iron per day, and the balance studies had shown that approximately 30 per cent of this, or 53 mg., was retained, and that 9.3 per cent, or 16 mg., was used immediately in hemoglobin formation, so that an abundance of metallic iron was being retained in the body.² With the administration of this amount of the drug there was a hemoglobin increase of such degree that the average gain at the end of twelve weeks was 1.82 Gm. per 100 c.c. of blood (Chart 1). Following this, as has been pointed out,¹⁷ there was a gradual reduction in the hemoglobin in spite of continued iron administration. It is well recognized that the rate of hemoglobin regeneration will depend on the degree of anemia as well as on the amount of iron administered. The anemia in this group of persons was mild and the gain of nearly 2 Gm. in the twelve-week period seems very satisfactory, although it is small in comparison with the results obtained in severe anemia.

A third group of 21 persons, with an average hemoglobin of 10.95 Gm., was given 3 Gm. (45 grains) of iron and ammonium citrates per day. This yields approximately 500 mg. of metallic iron per day; it has previously been shown that with this dosage 32.6 per cent of the iron was retained, but only 2 per cent of the amount administered was utilized immediately in hemoglobin formation in a group of 10 patients with an average hemoglobin level of 5.95 Gm.¹ This means that an average of 167.5 mg. of iron was being retained per day, 10.3 mg. was utilized in hemoglobin formation, and that 157.2 mg. was retained in addition to that used immediately in hemoglobin. With this dose of iron and ammonium citrates the average gain for the 21 patients was 1.78 Gm. at the peak of the response, a figure which is very similar to the response obtained with 1 Gm. of the same drug per day. The difference in the two groups lies in the fact that with 3 Gm. per day the peak of the hemoglobin production occurred in the eighth week instead of the twelfth week. The response was more rapid but no greater than with a smaller amount of iron (Chart 1). Following the peak in the hemoglobin response, there was a gradual regression during the following four weeks of observation in spite of continued iron administration.

Similar studies were carried out with small amounts of other simple iron salts for comparison with those obtained with iron and ammonium citrates. Reduced iron, 1 Gm. per day, was given to a group of 7 persons with an average hemoglobin of 11.36 Gm.; it produced a slightly greater increase in hemoglobin than did 1 Gm. of iron and ammonium citrates with the peak of the response in the tenth rather than in the twelfth week. Reduced iron is an insoluble preparation but one which is much higher in its metallic iron content than any other iron salt. As compared with 1 Gm. of iron and ammonium citrate, which contains approximately 170 mg. of metallic iron, 1 Gm. of reduced iron contains nearly 980 mg. of metallic iron so that the greater amount of iron compensates for its insolubility in producing the results shown in Chart 2.

Iron and sodium citrates, 1 Gm. per day, was administered to a group of 13 persons with an average hemoglobin level of 10.72 Gm. The results are shown in Chart 3. The response was somewhat less than with a similar dose of iron and ammonium citrates or reduced iron, but not significantly different.

Both clinical and experimental evidence points to the fact that ferrous salts are more effective than ferric salts when compared on the basis of their metallic iron content. This is substantiated by our results in which the administration of 0.36 Gm. (6 grains) of ferrous sulfate per day given to 11 persons with an average hemoglobin level of 10.58 Gm. produced a hemoglobin response comparable with those obtained with much larger doses of other iron salts (Chart 4). This amount of ferrous sulfate contains approximately 80 mg. of metallic iron as compared with 170 mg. in the smallest effective dose of iron and ammonium citrates and 980 mg. in reduced iron. It is comparable with the metallic iron content in 0.5 Gm. of iron and ammonium citrates which was shown to be ineffective.

It is interesting to note the similarity of the hemoglobin curve in every instance. As was pointed out in a previous communication, the hemoglobin increased to a peak; this was followed by a decline even though the same amount of iron was administered continuously. These persons were not fol-

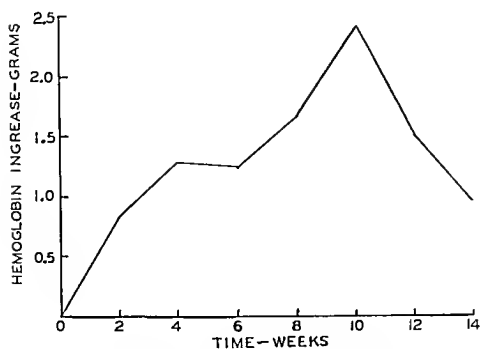


Chart 2.—Reduced iron, 1 Gm. daily.

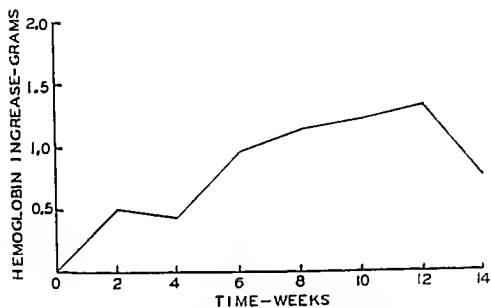


Chart 3.—Iron and sodium citrates, 1 Gm. daily.

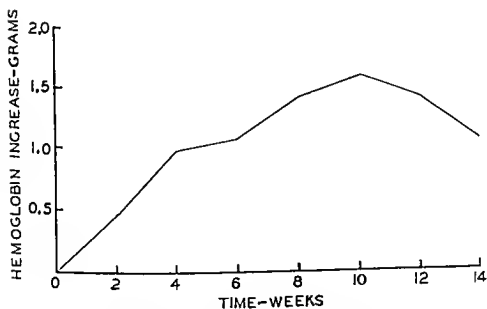


Chart 4.—Ferrous sulfate, 0.36 Gm. daily.

lowed for as long a period as was true in the preceding study, but the hemoglobin curve had essentially the same contour for the shorter period and supports the idea of a stimulating or catalytic effect of iron^{10, 18} on hemoglobin formation.

It is useless to speculate on the minimal effective dose of an iron salt because this will vary so greatly in individual patients. Even the commonly employed doses need to be increased in certain cases to procure an adequate response. Hence the results reported here are not intended to be an argument for a wholesale reduction in the amount of iron administered per day to these patients. The renaissance in iron therapy has been due in part to the administration of larger amounts of this mineral, and these larger dosages are advisable in cases of severe anemia and when it is impossible to follow the patient by frequent hemoglobin determinations. It has been shown that 80 per cent of the patients respond satisfactorily to smaller doses¹² so that in the case of mild degrees of anemia or as a maintenance dose in idiopathic hypochromic anemia smaller amounts should be satisfactory for adequate therapy. The results are presented as a comparison of the effectiveness of varying amounts of iron in different iron salts in comparable cases.

SUMMARY AND CONCLUSION

When varying doses of iron and ammonium citrates were employed in the treatment of mild anemias, it was found that 0.5 Gm. per day was too small to be of value. The response to 1 Gm. of the drug per day was satisfactory, although the hemoglobin increase was less rapid than with 3 Gm. of the same preparation per day. Reduced iron, 1 Gm. per day, and iron and sodium citrates, 1 Gm. per day, gave practically the same response as did 1 Gm. of iron and ammonium citrates. Ferrous sulfate, 0.36 Gm. (6 grains) per day, was as effective as 1 Gm. of the other drugs, although the metallic iron content is much lower.

REFERENCES

1. Fowler, W. M., and Barer, A. P.: Retention and Utilization of Orally Administered Iron, *Arch. Int. Med.* 59: 561, 1937.
2. Fowler, W. M., Barer, A. P., and Spielhagen, G. F.: Retention and Utilization of Small Amounts of Orally Administered Iron, *Arch. Int. Med.* 59: 1024, 1937.
3. Elvehjem, C. A., Siemers, A., and Mendenhall, D. R.: Effects of Iron and Copper Therapy on Hemoglobin Content of the Blood of Infants, *Am. J. Dis. Child.* 50: 28, 1935.
4. Brock, J. F., and Diamond, L. K.: Rickets in Rats by Iron Feeding, *J. Pediat.* 4: 442, 1934.
5. Barer, A. P., and Fowler, W. M.: The Effect of Iron on Phosphorus, Calcium, and Nitrogen Metabolism, *J. LAB. & CLIN. MED.* 26: 351, 1940.
6. Hurst, A. F.: A Case of Iron Encephalopathy, *Guy's Hosp. Rep.* 81: 243, 1931.
7. Meulengracht, E.: Large Doses of Iron in the Different Kinds of Anemia in a Medical Department, *Acta. med. Scandinav.* 58: 594, 1923.
8. Schulten, H.: Zur Behandlung hypochromer Anämien mit maximalen Eisendosen, *München med. Wchnschr.* 77: 355, 1930.
9. Heath, C. W.: Oral Administration of Iron in Hypochromic Anemia, *Arch. Int. Med.* 51: 459, 1933.
10. Brock, J. F.: The Relation Between the Hypochromic Anemias and Iron Deficiencies, *Brit. M. J.* 1: 314, 1937.
11. Fullerton, H. W.: The Treatment of Hypochromic Anemia With Soluble Ferrous Salts, *Edinburgh M. J.* 41: 99, 1934.
12. Wits, L. J.: Discussion on the Treatment of the Anemias, *Proc. Roy. Soc. Med.* 26: 607, 1932.

13. Moore, C. V., Arrowsmith, W. R., Welch, J., and Minnich, V.: Studies in Iron Transportation and Metabolism. IV. Observations on the Absorption of Iron From the Gastro-Intestinal Tract, *J. Clin. Investigation* 18: 553, 1939.
14. Newcomer, H. S.: A New Optional Instrument for the Determination of Hemoglobin, *J. Biol. Chem.* 55: 569, 1923.
15. Van Allen, C. M.: An Hematocrit Method, *J. LAB. & CLIN. MED.* 10: 1027, 1925.
16. Fowler, W. M., and Barer, A. P.: The Effect of Copper and Iron on Hemoglobin Regeneration, *J. LAB. & CLIN. MED.* 26: 832, 1941.
17. Fowler, W. M., and Barer, A. P.: Some Effects of Iron on Hemoglobin Formation, *Am. J. M. Sc.* In Press.
18. Whipple, G. H., and Robschheit-Robbins, F. S.: Blood Regeneration in Severe Anemia: Optimum Iron Therapy and Salt Effect, *Am. J. Physiol.* 92: 362, 1930.

BLOOD FINDINGS IN LATE PREGNANCY*

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BLOOD findings on pregnant women may not logically be compared with those on normal nonpregnant women. Frequently the differences found between normal and complicated pregnancies are extremely important; as, for example, in toxemia, anemia, or following sulfamidamide therapy. For obstetric work, therefore, standards or ranges based on findings on normal pregnant women should be used.

In Table I we present such data for the carbon dioxide and oxygen contents of the blood, the oxygen capacity, the red blood cell count and volume, and the hemoglobin. The table includes the maximum and minimum findings and the averages from a series of 30 dispensary patients, whose histories and physical examinations indicated that they were normal pregnant women. From these results we have calculated by distribution formulas the range which will include individual findings on all normal pregnant women 95 per cent of the time. For comparison we have included the normal nonpregnant values for these same determinations, compiled from findings published in the literature and accepted by various texts.

The oxygen and carbon dioxide determinations were made, with a few unavoidable exceptions, in duplicate, in a manometric apparatus after the method of Van Slyke and Neill,¹ using oxalated venous blood, drawn free flowing from the arm under oil. Agreement between the duplicates was good, averaging less than 2 per cent difference. The red blood cell counts and red blood cell volumes were determined on the same samples, in the usual manner. Hemoglobin readings were made by the Sahli method, using a standard of 13.8 mg. per 100 c.c. as 100 per cent, and were also calculated from the oxygen capacity by formulas.[†] Fresh blood was used throughout and all work was completed in from three to five hours from the time the specimen was drawn.

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[†]Hemoglobin, in grams per 100 c.c. of blood, is found by subtracting the 0.5 volume per cent of physically dissolved oxygen from the oxygen capacity, and multiplying the result by the factor 0.746.

In view of the numerous factors which may affect the oxygen content of venous blood, of which Keys² found individual variation the greatest, our values for carbon dioxide and oxygen content are remarkably consistent, showing somewhat less variation than others have reported. Our range and average are a little lower than those Keys found with nonpregnant women, and also less than Eastman's³ 11 volumes per cent at delivery, or Smith's⁴ average of 12.1 volumes per cent for 10 women in labor, before anesthesia or delivery.

TABLE I

	NO. DETERMI- NATIONS	AVERAGE	RANGE OF ACTUAL DETER- MINATIONS	STANDARD DEVIATION	CALCULATED RANGE* (IN- CLUDES 95 PER CENT OF ALL NORMAL PREGNANT DETERMINA- TIONS)	NORMAL RANGE FOR NONPREG- NANT WOMEN TAKEN FROM THE LITERATURE
CO ₂ content in volume per cent	30	47.66	38.66-55.04	3.58	40.50-54.82	45-60
O ₂ content in volume per cent	30	6.90	2.80-12.13	2.18	2.55-11.25	11-17.6
O ₂ capacity in volume per cent	30	15.25	12.13-19.37	1.79	11.65-18.85	17-20
Erythrocytes per c.mm.	30	3.78	2.94- 4.79	0.53	2.71- 4.84	4.25- 5.00
Red blood cell volume in per cent	29	28.50	22.2-33.0	2.73	23.04-33.96	35-45
Hemoglobin by O ₂ ca- pacity	30	11.00	8.68-14.08	1.35	8.30-13.70	13.0-15.7
Gm. per 100 c.c.						
Hemoglobin by Sahli	24	11.00	8.42-12.97			13.8
Gm. per 100 c.c.						

*Two times the standard deviation added to and subtracted from the average gives the range which includes all individual determinations 950 times out of 1,000.

The oxygen capacity determination is more exact, since it is not affected by the factors which tend to vary the content, and is the same for both venous and arterial blood. Here again pregnancy causes a definite lowering from the generally accepted standards. For normal nonpregnant women Peters and Van Slyke⁵ give 17 to 21 volumes per cent oxygen capacity; Keys, 17 to 18; and Todd and Sanford,⁶ 18 to 24 for both men and women, men being conceded to average higher than women. Our average of 15.25 volumes per cent agrees well with Eastman's 15.4 at delivery, without anesthesia, and is but slightly lower than Smith's average of 16.8 for women in labor.

Oxygen capacity determinations are particularly practical following sulfanilamide therapy. This is illustrated by Table II, where we have presented a brief survey of the laboratory findings on 8 selected patients. Five of these, Nos. 1, 2, 3, 6, and 8, show a serious fall in oxygen capacity to a level below the lower limits, 11.65 volume per cent, of the normal pregnant range—3 by more than 2 volume per cent. In Case 2, even as low a concentration of sulfanilamide as 2.7 mg. per 100 c.c. of blood was sufficient to depress the oxygen capacity. Cases 4 and 7 show declines that were checked by blood transfusions and partial withdrawal of the drug. This decline in oxygen capacity only occasionally follows the use of the drug, but it is always a possibility that should never be

neglected. In the cases where it does occur, the oxygen-carrying power of the cells is apparently blocked, so that an oxygen tent is powerless to relieve the situation. This conclusion is supported by the fact that as the oxygen capacity falls different hemoglobin methods fail to agree, the colorimetric methods, either Sahli or alkaline hematin, invariably giving higher results. Further, there is no correlation between the blood count and the oxygen capacity. Withdrawal of the drug, supported by transfusions, restores the oxygen capacity and the agreement between the hemoglobin findings.

Another effect, illustrated in Cases 2, 4, 5, and 7, is the depression of the carbon dioxide content of the whole blood. This does not parallel the oxygen capacity fall, nor is it exactly proportional to the concentration of the drug in the blood, but it is relieved by withdrawal of the drug or by blood transfusions. No significance has so far been attached to it.

The per cent of oxygen saturation is varied not only by all the factors which affect the content, but also by those which affect the capacity. As is to be expected, our range is quite wide, and little significance can be attached to individual findings. The tendency to lower averages, however, is interesting. Smith reported 71.1 per cent for women in labor; Eastman, 71 per cent at delivery, with no anesthesia; and Keys, 62.8 per cent for nonpregnant women. We find an average of only 44.9 per cent for late pregnancy. Although the red blood cell count varies quite widely and is of less diagnostic value here than in other circumstances, it is consistently lower than that of nonpregnant normal women, averaging 3.77. The red blood cell volume, however, is quite consistent, averaging 28.5 per cent, as opposed to the nonpregnant 40 per cent. The hemoglobin derived from the oxygen capacity naturally parallels those values, and shows little variation. The Sahli findings likewise are very consistent and agree remarkably with the other hemoglobin values, averaging the same. While the findings on a given individual do not always agree, it is worthy of note that no Sahli readings fall outside the calculated range established by the oxygen capacity method for hemoglobin.

There is less interrelation between these different blood findings than might be anticipated. The oxygen and carbon dioxide contents might seem to be inversely proportional, but upon applying Pearson's correlation formulas we find the relation just fails of significance. Neither is there a direct relation between the red blood cell count and red blood cell volume, probably due to variation in the size of the red blood cells, even when applying Pearson's less exact rank formulas, which take into account only the order of magnitude instead of exact values. The oxygen capacity and red blood cell volume did show a bare significance, but in view of the short series reported, this can only be noted as a tendency which might be confirmed in a longer series.

Apparently neither age nor the number of children affects the blood findings. Seven individuals reported were over 25, two were 40, yet none show any significant variation from the general average. Similarly, 11 of the 30 had 2 or more children, one 5, one 6, and one 9; yet these, too, agree with the general averages.

SUMMARY

Tables of blood findings, including the calculated ranges for normal cases of late pregnancy, are presented.

The practical value of laboratory work for patients on sulfanilamide or allied drug therapy is illustrated with a few selected cases, showing a serious fall in oxygen capacity unrelated to the red blood cell count.

Lack of correlation is demonstrated between the widely varying red blood cell counts and the more uniform red blood cell volumes, while a tendency toward correlation is indicated between the oxygen capacity and the red blood cell volume.

A rough parallel is shown between the colorimetric Sahli hemoglobin readings and those calculated from the oxygen capacity, and thus between the Sahli readings and the oxygen capacity.

REFERENCES

1. Van Slyke, D. D., and Neill, J. M.: The Determination of Gases in Blood and Other Solutions by Vacuum Extraction and Manometric Measurement, *I. J. Biol. Chem.* 61: 523, 1924.
2. Keys, Ancel: The Oxygen Saturation of the Venous Blood in Normal Human Subjects, *Am. J. Physiol.* 124: 13, 1938.
3. Eastman, N. J.: V. The Role of Anesthesia in the Production of Asphyxia Neonatorum, *Am. J. Obst. & Gynec.* 31: 563, 1936.
4. Smith, C. A.: The Effect of Obstetrical Anesthesia Upon the Oxygenation of Maternal and Fetal Blood With Particular Reference to Cyclopropane, *Surg., Gynec., & Obst.* 69: 584, 1939.
5. Peters, J. P., and Van Slyke, D. D.: Quantitative Clinical Chemistry, Baltimore, 1932, Williams & Wilkins Co., Vol. II, Table 39, p. 544.
6. Todd, J. C., and Sanford, A. H.: Clinical Diagnosis by Laboratory Methods, Philadelphia, 1936, W. B. Saunders Co., p. 736.

CORRELATED HEMATOLOGIC, PHYSICAL, AND CHEMICAL STUDIES OF THE BLOOD AND STROMA IN POLYCYTHEMIA AND BEEF EMBRYO*

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INVESTIGATIONS of the blood in hemolytic¹ and pernicious anemias² have shown marked alterations in erythrocyte composition, and chemical study of posthemolytic residue, or "stroma," prepared from the blood of dogs with experimental disulphide anemia has proved that abnormalities in the chemical structure of the red blood cell may be induced by anemia.³ Human polycythemia offers a striking hematologic contrast to the anemias; furthermore, it provides an opportunity to secure sufficient human blood for complete hematologic, physical, and chemical investigations, together with the simultaneous preparation and study of the erythrocyte stroma.

Our present objective has been to make a coordinated and as thorough investigation of the blood in polycythemia as possible. The detailed study of a few

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specific cases has been chosen rather than less exhaustive observations upon a larger number of patients. In order to interrelate the hematologic, physical, and chemical characteristics of the blood with that of the stroma or fixed framework of the erythrocyte, a relatively large quantity of blood, from the standpoint of the human subject, must be obtained, despite the use of microchemical methods of analysis. From 30 to 50 ml. of blood are required for all the hematologic, physical, and chemical measurements. An additional quantity (250 to 500 ml.) of blood must be secured for the stroma preparation.* From 5 to 10 Gm. of erythrocyte posthemolytic residue or stroma are required for lipid and amino acid analyses.

The patients of this study have been under clinical observation and treatment for a number of years, and inasmuch as the withdrawal of blood at periodic intervals was the procedure used to control the polycythemia, it offered the first opportunity to make this type of investigation with human beings.

EXPERIMENTAL

Two established cases of primary polycythemia vera and one of cardiac origin were studied. Subject J. S.† (male, aged 49 years) had been under observation for over ten years. Treatment with phenylhydrazine and x-ray had been used, but not in the three years prior to the time at which these studies were made. Subject P. H.‡ (male, aged 31 years) exhibited a mild polycythemia vera, with a red blood cell count never above 7.5 million per cubic millimeter. Subject A. D.‡ (male, aged 31 years) suffered from a congenital heart deficiency and was reported to have been cyanotic since infancy. All three patients were being treated by periodic venesection during the time these studies were made.

Samples of blood for chemical analysis and stroma preparation were removed by venepuncture. Determinations of red blood cell count, hemoglobin, hematocrit, diameter, specific gravity, and water content were made; from these, corpuscular volume, weight, thickness, and hemoglobin and water content were calculated. The chemical data included mineral analyses (sodium, potassium, chloride, calcium, and phosphorus) of the serum and red blood cells; the lipid distribution (total, free, and esterified cholesterol, total phospholipid, lecithin, cephalin, sphingomyelin, cerebrosides, and neutral fat) of the plasma and erythrocytes; and the amino acid and lipid partition of the stroma prepared from the erythrocytes. The hematologic and chemical methods have been outlined in previous studies from this laboratory,⁴⁻⁶ as has the procedure developed for the preparation of the posthemolytic residue.⁷

*Preparation of stroma involves separation of the fixed framework of the erythrocyte. The erythrocytes are separated by centrifugation and washed then hemolyzed in physiologic saline. The washed erythrocytes are run through resuspended in a buffer (pH 5.0 to 5.5). The material is then again placed in 20 volumes of buffer and run through a refrigerator. This suspension is ground in a ball mill in a vacuum oven and powdered.⁷

†We wish to thank Dr. E. A. Sharp of Parke, Davis & Co. and the staff of the anemia clinic of Harper Hospital for their cooperation in making accessible these cases for study, and for their assistance in the collection of the blood samples.

‡We appreciate the courtesy of Dr. Warren B. Cooksey and Dr. Hugo A. Freund in arranging and assisting with the studies on patient P. H.

RESULTS AND DISCUSSION

The hematologic and physical characteristics of the erythrocytes are given in Table I. The average red blood cell of the mild polycythemia vera (P.H.) is of normal size, shape, and weight, in agreement with general literature reports; the volume and weight in the more severe case (J.S.) show some decrease. Both cases demonstrate a decreased quota of hemoglobin per cell. In contrast, the erythrocyte of the cardiac polycythemia (A.D.) exhibits a full quota of hemoglobin and increased volume and weight owing in part to elevated mineral and lipid contents, but chiefly to an augmented water content. Values indicative of the weight of the nonhemoglobin protein of the cell are low for polycythemia vera (1.1 and 2.1 Gm. $\times 10^{-12}$) but normal in the cardiac case (3.3 Gm. $\times 10^{-12}$). Comparable, but slightly lower values, are obtained from the difference between the total nitrogen of the cryoscopically dried cell and that accounted for by the hemoglobin and lipid.

TABLE I

HEMATOLOGIC AND PHYSICAL CHARACTERISTICS OF ERYTHROCYTES IN POLYCYTHEMIA

	NORMAL	POLYCYTHEMIA		
		VERA		CARDIAC
		J. S.	P. H.	A. D.
Hemoglobin (Gm./100 c.c.)	15.4	17.5	15.6	24.3
Red blood cells (millions/c.mm.)	5.4	7.2	6.6	7.6
Hematocrit (per cent)	44	67	60	82
Corpuscular:				
Volume (c. μ)	86	82	90	102
Diameter (μ)	7.7	7.7	7.7	7.6
Thickness (μ)	1.8	1.8	1.9	2.2
Weight ($\times 10^{-12}$ Gm.)	95	88	97	110
Hemoglobin ($\times 10^{-12}$ Gm.)	29	21	23	30
Water ($\times 10^{-12}$ Gm.)	63	63	69	75
Mineral ($\times 10^{-12}$ Gm.)	0.6	0.6	0.8	0.8
Lipid ($\times 10^{-12}$ Gm.)	0.4	0.6	0.6	1.1
Nonhemoglobin protein ($\times 10^{-12}$ Gm.)	1.9	--	1.3	2.1
Undetermined weight ($\times 10^{-12}$ Gm.)	3.0	2.1	1.1	3.3
Nonhemoglobin protein/lipid ratio	4.9	--	2.3	2.0

The mineral analyses of the serum and erythrocytes are given in Table II. The values for serum water, protein, sodium, potassium, chloride, calcium, and phosphorus are within normal limits. Values for the mineral and lipid composition of the erythrocytes are given in terms of average corpuscular concentration, rather than on the basis of a unit weight of cells, in order to compare the composition of the individual cell in the various conditions.^{1,4} Only in cardiac polycythemia were abnormalities displayed in the erythrocyte content of minerals; increases were found in sodium, chloride, and water. Calculation on the basis of milliequivalents per liter of cell water further demonstrates the elevated chloride and sodium values, and, in addition, a compensatory decrease in potassium.

Data on the lipid distributions of the plasma, cells, and stroma are presented in Table III. A reduced free cholesterol in the plasma of polycythemia vera was a consistent finding. An increased plasma phospholipid with a significantly higher proportion present as cephalin was observed in the patient with cardiac polycythemia. Although Boyd has reported no disturbance in the concentration of plasma lipids in polycythemia,⁸ inspection of his data reveals trends similar

to those noted above; i.e., a reduced free cholesterol in polycythemia vera and an elevated phospholipid in secondary polycythemia.

The data demonstrate that the increase in total corpuseular lipid in polycythemia vera is due mainly to an elevated total phospholipid, which is reflected in the cephalin and sphingomyelin fractions. In the patient with cardiac disorder the marked augmentation of corpuseular lipid is accompanied by corresponding increases in free cholesterol and neutral fat as well as total phospholipid. It seems significant, however, that the major increase occurs in the phospholipid and is reflected in all three fractions, cephalin, sphingomyelin, and lecithin.

TABLE II
MINERALS OF SERUM AND ERYTHROCYTES IN POLYCYTHEMIA*

	NORMAL†	POLYCYTHEMIA		
		VERA		CARDIAC
		J. S.	P. H.	A. D.
Plasma protein (Gm./100 c.c.)	7.1	7.2	6.9	8.1
Serum:				
Sodium (meq./liter)	140	140	143	143
Potassium (meq./liter)	4	7	4	7
Chloride (meq./liter)	103	97	101	103
Water (Gm./liter)	944	932	935	936
Erythrocyte:				
Sodium (meq. $\times 10^{-13}$ /cell)	15	21	20	44
Potassium (meq. $\times 10^{-13}$ /cell)	80	96	133	96
Chloride (meq $\times 10^{-13}$ /cell)	45	45	51	70
Water (Gm. $\times 10^{-12}$ /cell)	63	63	69	75
Serum:				
Sodium (meq./liter of water)	148	150	153	153
Potassium (meq./liter of water)	4	8	4	7
Chloride (meq./liter of water)	100	104	108	110
Erythrocyte:				
Sodium (meq./liter of water)	25	31	27	54
Potassium (meq./liter of water)	144	141	179	117
Chloride (meq./liter of water)	72	66	69	86

*Values for calcium, inorganic, and acid-soluble phosphorus, in serum and erythrocytes, were normal.

†Values from previous studies^{2,4}

The increased phospholipid, particularly the cephalin and sphingomyelin fractions, is in striking contrast to the characteristic changes of the erythrocyte composition in severe anemia;^{1,2} it is similar, however, to the chemical alterations induced in the red blood cells by liver therapy in pernicious anemia during the period of active reticulocyte response.^{2,9} There seems to be no evidence, therefore, of an accumulation of old cells exhibiting fatty degeneration; rather, the mass presence of young cell forms and active bone marrow stimulation.* These findings are in agreement with hematologic observations of the presence of immature red blood cell forms in the circulation^{10,11} and the histologic evidence of increased erythroblastic activity of the bone marrow in polycythemia.^{12,13}

The augmented lipid content of the erythrocyte may be indicative of a greater amount of fixed lipid structure, inasmuch as experimental data have

*An increased span for beginning and complete hemolysis by hypotonic sodium chloride solutions gave evidence of the presence of cells of varied ages, particularly immature forms. The periodic venesection may have had some effect upon bone marrow activity and the type of erythrocytes released into circulation.

TABLE III
DISTRIBUTION OF LIPIDS IN BLOOD AND STROMA IN POLYCYTHEMIA

	PLASMA (MG./100 G.C.)				ERYTHROCYTE (MG. X 10 ⁻¹² /CELL.)				STROMA (PER CENT DRY WEIGHT)						
	NORMAL HUMAN*	POLYCYTHEMIA			NORMAL HUMAN	POLYCYTHEMIA			NORMAL HUMAN*	POLYCYTHEMIA			NORMAL*	EMBRYO†	
		J. S.	P. H.†	CARDIAC		J. S.	P. H.†	CARDIAC		J. S.‡	P. H.†	CARDIAC			
															VERA
R. B. C. (millions/c.mm.)															
Total lipid	750	721	420	796	399	561	8.9	7.2	14.1	18.4	20.0	21.5	25.6	19.5	
Cholesterol:															
Free	82	48	37	73	92	109	136	109	2.3	4.9	5.2	5.7	6.9	5.1	
Esters	254	198	180	322	30	39	0	0	0.5	0.1	0.7	0.5	0.6	0.4	
Phospholipid:															
Total	189	245	130	303	233	359	407	359	9.6	10.0	12.0	12.6	16.0	11.9	
Lecithin	99	158	50	--	70	61	74	61	2.0	3.6	2.4	3.2	--	2.2	
Cephalin	55	66	43	210	173	218	244	218	4.6	3.4	4.5	6.9	7.8	6.3	
Sphingomyelin	35	21	37	--	45	82	89	82	3.1	3.0	5.1	2.5	--	3.1	
Cerebrosides	15	11	0	27	43	35	76	35	1.7	1.6	2.1	2.5	--	1.6	
Neutral fat	210	220	80	71	5	19	13	19	0	1.8	0	0.1	2.1	0.5	

*Values from previous studies.^{2, 4, 5, 14}

†Average of 2 samples.

‡Average of 6 samples.

§Average of 4 samples.

shown that practically all of the lipid in the intact erythrocyte is combined with the stroma.¹⁴ The posthemolytic residue prepared from the erythrocytes in polycythemia exhibited an elevation of total lipid, chiefly cholesterol (Table III). An augmented phospholipid content was found in the preparations from P. H. and A. D. and was reflected in the sphingomyelin and cephalin fractions, respectively. The differences between cells and stroma in the phospholipid partition indicate that a portion of that present in the whole cell is not combined with the stroma. These lipid anomalies are diametrically different from those found in the red blood cell stroma of dogs subjected to experimental N-propyl disulfide anemia, in which reductions of total lipid, phospholipid, and cholesterol were observed.³

The lipid distribution of the posthemolytic residue of embryonic beef erythrocytes (Fig. 6) has been included (Table III) in view of suggestions that polycythemia vera may be analogous to fetal polycythemia.¹⁵ No similarities between the lipid abnormalities of the embryonic beef and polycythemia vera erythrocytes are evident. In fact, the embryonic preparations, in comparison with stroma from erythrocytes of adult beef, exhibit lower total lipid, free cholesterol, and phospholipid, differences which are suggestive of the anemias, and recall the hematologic similarities of fetal and pernicious anemia erythrocytes.¹⁵

TABLE IV

AMINO ACID ANALYSES OF ERYTHROCYTE POSTHEMOLYTIC RESIDUE IN
POLYCYTHEMIA AND BEEF EMBRYOS
(MILLIMOLS PER 100 GM. OF PROTEIN*)

	NORMAL HUMAN	POLYCYTHEMIA			BEEF	
		VERA		CARDIAC ^c	NORMAL [†]	EMBRYO [‡]
		J. S. [‡]	P. H.	A. D.		
Arginine	27.6	26.6 ± 0.6	27.4	19.5	29.3	29.0 ± 2.7
Histidine	13.5	11.7 ± 1.7	8.7	13.5	12.3	11.5 ± 1.3
Lysine	26.0	24.8 ± 2.0	26.8	21.0	24.0	31.0 ± 2.4
Cysteine	7.5	9.9 ± 0.8	8.0	8.8	7.4	9.8 ± 0.8
Tyrosine	14.9	15.3 ± 0.6	15.6	--	16.0	16.9 ± 2.0
Tryptophane	5.9	5.8 ± 0.3	5.6	--	5.9	5.7 ± 0.5
Total nitrogen (per cent)	13.0	13.1 ± 0.4	12.6	13.7	13.8	13.7 ± 0.2

*Analyses on alcohol ether extracted preparation

[†]Values from previous studies.⁶

[‡]Average of 4 preparations with average deviation from the mean.

[§]Average of 5 preparations with average deviation from the mean

The protein fraction of the posthemolytic residue has been analyzed for six amino acids: arginine, histidine, lysine, cysteine, tyrosine, and tryptophane. Since these amino acids do not have uniform molecular weights or nitrogen contents, the analyses are expressed in terms of millimols per 100 Gm. of protein in Table IV. In polycythemia vera less histidine was found than is present in normal stroma; in the severe case (J. S.) the decreased histidine was accompanied by an increase in cysteine content. The stroma protein from the cardiac polycythemia erythrocytes contained more cysteine but less arginine and lysine than normal.

If 35,000 is taken arbitrarily as the molecular weight of the stroma protein, then a deviation from the normal of 2.86 millimols of an amino acid per 100 Gm. of protein represents a variation of 1 Gm. mol of that amino acid per 35,000 Gm. of protein, and may be considered a significant difference. By this criterion only

cysteine differs significantly (one-half to 1 mol) in the preparations of J.S., while histidine is lowered by 1 to 2 Gm. mols per 35,000 Gm. protein in the stroma of P.H. In the cardiac case, both arginine and lysine are diminished significantly (2 mols).

TABLE V
SIGNIFICANCE OF DIFFERENCES* IN AMINO ACID ANALYSIS
(MILLIMOLS PER 100 GM. OF PROTEIN)

	POLYCYTHEMIA			EMBRYONIC BEEF
	VERA		CARDIAC	
	J. S.	P. H.	A. D.	
Arginine	-0.9	-0.1	-8.0	-0.3
Histidine	-1.8	-1.8	0	-0.7
Lysine	-1.2	+0.8	-5.0	+7.0
Cysteine	+2.4	+0.5	+1.3	+3.3
Tyrosine	+0.4	+0.7	-	+0.9
Tryptophane	0	-0.3	-	-0.2

*A variation from the normal of 2.86 millimols per 100 Gm. of protein is equivalent to 1 mol of an amino acid per 35,000 Gm. of protein (the assumed molecular weight), and therefore, a difference of this magnitude or larger may be considered significant.

The amino acid analyses of the embryonic beef erythrocyte residues* exhibit significant differences in comparison with preparations from adult beef erythrocytes, but differences which are not similar to those displayed in polycythemia. Outstanding is the increase of the lysine fraction by 2 mols, and the cysteine by 1 mol.

This evidence of abnormalities in the amino acid composition of stroma in polycythemia is unique, in view of previous studies which have shown that posthemolytic residue preparations from the erythrocytes of 5 mammalian species were practically identical in amino acid composition.⁶ Furthermore, no alterations were found in the amino acid composition of red blood cell stroma of dogs with experimental disulfide anemia.³

SUMMARY

Correlated hematologic, physical, and chemical investigations of the blood plasma and erythrocytes have been made in two cases of polycythemia vera and one of secondary polycythemia. These studies have been accompanied by detailed lipid and amino acid analyses of the erythrocyte posthemolytic residue or stroma. Analyses on the stroma of embryonic beef erythrocytes are included for comparison.

The data indicate significant alterations not only in the composition of the intact erythrocytes but also in the lipid and amino acid constitution of their so-called fixed structure or stroma. Increased corpuscular total lipid and phospholipid, accompanied by corresponding increases of cephalin and sphingomyelin were the salient characteristics. The posthemolytic residues prepared from the erythrocytes exhibited an elevation of total lipid and cholesterol. The data indicate, however, that not all the augmented phospholipid evident in the cell in polycythemia is bound with the stroma.

*These differences in composition of the erythrocyte residue of embryo bloods from that of the normal adult have suggested a study which is now in progress: the pattern of the chemical composition of tissues during growth.

No similarities were evident between the stroma from the erythrocytes of beef embryos and polycythemia in their lipid anomalies.

Significant, but dissimilar, differences were found in the amino acid composition of the erythrocyte stroma protein from polycythemia vera, cardiac polycythemia, and embryonic beef preparations.

The cardiac polycythemia exhibited more marked and extensive changes in the mineral and lipid composition of the erythrocyte as well as the lipid and amino acid make-up of the posthemolytic residue.

These data in polycythemia add further evidence to those in the anemias that the stage of maturation of the erythrocyte may be reflected closely in its lipid composition and that of the posthemolytic residue.

REFERENCES

1. Erickson, B. N., Williams, H. H., Hummel, F. C., Lee, P., and Macy, I. G.: *J. Biol. Chem.* 118: 569, 1937.
2. Williams, H. H., Erickson, B. N., Bernstein, S., Hummel, F. C., and Macy, I. G.: *J. Biol. Chem.* 118: 599, 1937.
3. Williams, H. H., Erickson, B. N., Beach, E. F., and Macy, I. G.: *J. LAB. & CLIN. MED.* 26: 996, 1941.
4. Erickson, B. N., Williams, H. H., Hummel, F. C., and Macy, I. G.: *J. Biol. Chem.* 118: 15, 1937.
5. Erickson, B. N., Avrin, I., Teague, D. M., and Williams, H. H.: *J. Biol. Chem.* 135: 671, 1940.
6. Beach, E. F., Erickson, B. N., Bernstein, S. S., Williams, H. H., and Macy, I. G.: *J. Biol. Chem.* 128: 339, 1939.
7. Bernstein, S. S., Jones, R. L., Erickson, B. N., Williams, H. H., Avrin, I., and Macy, I. G.: *J. Biol. Chem.* 122: 507, 1938.
8. Boyd, E. M.: *Canad. M. A. J.* 34: 532, 1936.
9. Williams, H. H., Erickson, B. N., Bernstein, S. S., and Macy, I. G.: *Proc. Soc. Exper. Biol. & Med.* 45: 151, 1940.
10. Isaacs, R.: *Arch. Int. Med.* 31: 289, 1923.
11. Reznikoff, P.: *Bull. New York Acad. Med.* 15: 311, 1939.
12. Harrop, G. A., Jr.: *Medicine* 7: 291, 1938.
13. Weber, F. P.: *Polyeythemia, Erythrocytosis and Erythraemia*, London, 1921, H. K. Lewis & Co., Ltd.
14. Erickson, B. N., Williams, H. H., Bernstein, S. S., Avrin, I., Jones, R. L., and Macy, I. G.: *J. Biol. Chem.* 122: 515, 1938.
15. Wintrobe, M. M., and Shumacker, H. B., Jr.: *J. Clin. Investigation* 14: 837, 1935.

THE CHEMISTRY OF INFECTIOUS DISEASES*

I. POLAROGRAPHIC STUDY OF BLOOD SERA IN EXPERIMENTAL PNEUMONIA IN DOGS

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BRDICKA¹ reported that carcinomatous serum gave a typical polarographic curve, differing from that given by normal serum, by which early cases of malignant growth could be detected. Waldschmidt-Leitz,² in a study of over 500 cases, confirmed this observation. A check on the polarographic findings by clinical methods revealed that less than 10 per cent of the polarographic diagnoses were incorrect. Of these the wrong positive diagnoses were always of a deep-seated inflammatory nature, which prompted us to investigate the influence of severe pneumoecal inflammations on the normal polarographic curve of serum.

EXPERIMENTAL

All dogs were infected† by the method of Terrell, Robertson, and Coggeshall³ by the direct introduction of type I pneumococcus culture‡ into one lobe of the lung after the dog's body temperature had been lowered 3° to 4° F. by the subcutaneous injection into the groin of a saline solution of 6 mg. of morphine sulfate per kilogram of body weight.

After infection periodic samples of 5 to 8 c.c. of blood from either the cephalic or the external jugular veins were taken every three to four hours for the first thirty hours after infection, later twice daily, and then once until the dog had recovered or died. The sera were obtained in the usual way from the clotted and centrifuged blood.

Plenty of water was available to the animals at all times. A sufficient amount of our usual dog diet (horse meat and Purina Dog Chow) was given once a day. During the first and second days after infection the dogs often did not eat food.

A Heyrovsky self-recording polarograph was used throughout this work. The buffer solution for the serum analyses was similar to the one recommended by Brdicka.¹ It was prepared as follows:

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†We gratefully acknowledge the invaluable help and the unstinted cooperation we received in this study from Dr. W. Harry Feinstone of this laboratory. Dr. Feinstone, while actively studying related phases of pneumococcal infection in dogs, kindly took over the culturing of the pneumococci and the actual infection of the dogs, and followed the progress of the disease systematically with x-ray photographs.

‡Furnished through the courtesy of Dr. O. H. Robertson, of the University of Chicago.

10 c.c. of a cobaltic hexammino-chloride solution (0.2776 Gm. in 100 c.c. H ₂ O; decomposes within a few hours)
5 c.c. 1 N ammonium chloride
15 c.c. 1 N ammonium hydroxide
70 c.c. water
100 c.c. solution

In the analyses of whole serum, 0.45 c.c. of water was added to 0.3 c.c. of serum, and 0.1 c.c. of this diluted serum solution was electrolyzed in the presence of 10 c.c. of the cobalt buffer. The current-voltage curves were recorded in all cases from -0.9 to -1.7 volts. The sensitivity of the instrument was set at $\frac{1}{200}$, with an accumulator voltage of 4.0 volts. The dropping rate of the mercury electrode was 2.8 seconds per drop into the buffer solution at zero potential.

A variation from this procedure, also recommended by Brdicka¹ and referred to in this paper as "peptone fraction," consisted in the denaturation of 0.4 c.c. of serum with 1 c.c. of 1 N potassium hydroxide for exactly forty-five minutes, then precipitation of the macropoteins with 1 c.c. of 20 per cent sulfosalicylic acid and filtration after ten minutes' standing. One-half cubic centimeter of the filtrate was added to 5 c.c. of the cobalt buffer for electrolysis. The current-voltage curves were recorded as described under whole serum, except that the sensitivity was set at $\frac{1}{100}$.

The solid content of serum was determined by drying exactly 1 c.c. of serum in a weighing flask to constant weight in a vacuum desiccator over phosphorus pentoxide at room temperature.

TABLE I

AVERAGE, MAXIMUM, AND MINIMUM VALUES OF THE DIFFERENCES IN POLAROGRAPHIC WAVE HEIGHT BETWEEN -1.65 AND -1.06 VOLTS OF FIVE DOGS INFECTED WITH TYPE I PNEUMOCOCCUS

Differences between -1.65 and -1.06 volts in centimeters.

DOG NO.	NO. OF DETERMINATIONS	NORMAL DOGS			INFECTED DOGS
		AVERAGE	MAXIMUM	MINIMUM	MINIMUM
I	10	2.19	2.28	1.96	1.89
II	10	2.13	2.29	1.96	1.86
III	16	1.99	2.44	1.89	1.68
VI	18	2.15	2.35	1.97	1.69
VIII	3	2.04	2.14	1.96	1.68

RESULTS AND DISCUSSION

A polarographic wave of the whole serum of Dog VI prior to infection is recorded in Fig. 1, curve I, while Fig. 1, curve II, is the corresponding curve of the serum at the height of infection. It can be seen from the two figures that the minimum at -1.65 volts in the case of the normal serum is higher in relation to the flat part of the curve at -1.06 volts than the corresponding minimum of the infected serum. We selected, as a base line, the value at -1.06 volts, even though it is on a slope, since it represents the maximum of the current-voltage curve of the cobalt-buffer solution in the absence of proteins, and because it is very easily reproducible for a given serum. If this difference is plotted against

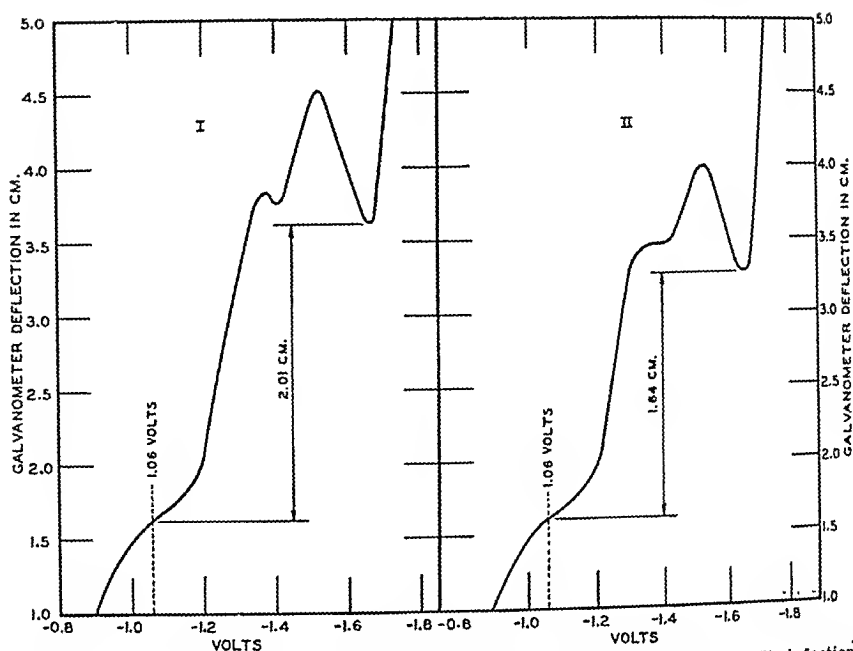


Fig. 1.—Polarograms of whole dog sera before (curve I) and after (curve II) infection with type I pneumococcus.

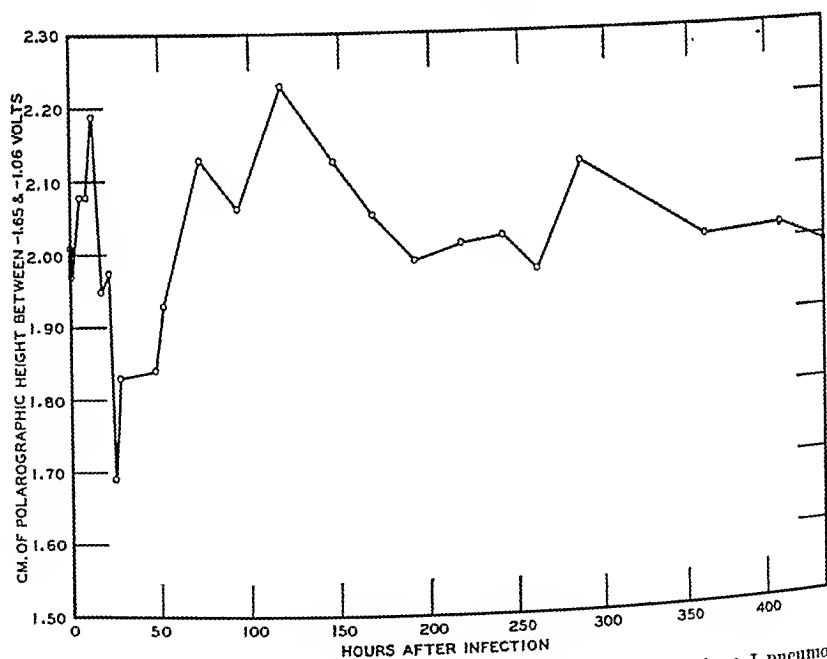


Fig. 2.—Polarographic wave height of whole dog sera after infection with type I pneumococcus

time, i.e., hours after infection, a curve such as shown in Fig. 2 for Dog VI is obtained. During the first thirty to fifty hours after infection, a sharp drop in the wave height occurred in all the five infected dogs studied. The return to normal values is then very rapid. However, the decrease is not uniform and loses much of its value due to the fluctuations in the wave height of the normal sera of the same dog, as shown in Table I, which summarizes the distances in

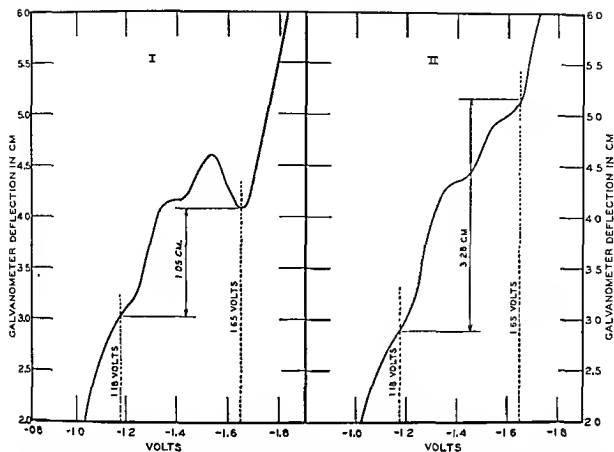


Fig 3—Polarograms of the filtrates of potassium hydroxide denatured, sulfosalicylic acid precipitated dog sera before (curve I) and after (curve II) infection with type I pneumococcus

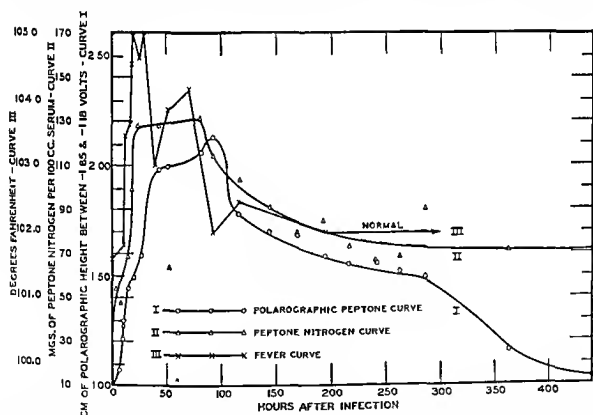


Fig. 4.—Changes in polarographic wave height (curve I) and nitrogen content (curve II) of the peptone fraction of sera (i.e., filtrate from potassium hydroxide denaturation and sulfosalicylic acid precipitation) and body temperatures (curve III) of the same dog with time after infection with type I pneumococcus

centimeters between -1.65 volts and -1.05 volts of the five infected dogs at the height of the illness, as well as the averages, maximum and minimum values of the corresponding normal blood samples.

The decrease in wave height, nevertheless, is real, reproducible, and undoubtedly represents some change in the serum with infection. Probably it is due to a decrease in proteic sulfhydryl and disulfide groups, i.e., a decrease in cystine (and cysteine) in peptide linkage, for we confirmed Brdicka's observa-

tion⁴ that proteins with little or no cystine sulfur (protamine and gelatin) as well as free cystine and cysteine, give no typical protein double wave in the trivalent cobalt buffer, while glutathione produces slight, and cystine-rich proteins a pronounced, double wave. The addition of free cystine either to serum or to crystalline serum albumin, buffered with trivalent cobalt, also does not increase the wave height from that given by the same amount of serum or albumin without the cystine.

While the polarograms of whole sera decrease in wave height with infection, probably indicating concentration changes, the current-voltage curves of the peptone fraction not only increase in wave height, but also undergo a pronounced alteration in the slope of the curves. This is clearly demonstrated in Fig. 3, curves I and II, which represent enlarged reproductions of the polarograms of normal and infected peptone fractions, respectively, of blood sera of Dog VI. It should be observed that the most pronounced changes in appearance of the curves occur at -1.65 volts. With an increase in infection the pronounced minimum of the double wave of normal serum gradually flattens out until, during severe infections, the curve at -1.40 volts becomes almost a straight line sloping upward. As the infection recedes, the height of the wave decreases until, upon complete recovery, the typical curve of a normal serum is obtained. If the difference in the peptone wave height between -1.65 volts and -1.18 volts is plotted against time after infection, a curve such as shown in Fig. 4, curve I (Dog VI), is obtained. The reason for choosing -1.18 volts as a base line, even though it falls on a slope, is that the value is constant in all normal dogs and changes very little during the entire period of infection. The peptone-fraction graphs of seven infected dogs show the same general tendency as the one represented in Fig. 4, curve I, for Dog VI, the only differences being that the absolute values of the peaks in the wave height, as well as the exact time after infection at which the peaks occur, vary with each dog. After recovery from the illness all seven dog curves return to their normal values, which lie between 0.85 and 1.25 cm. wave height difference (1.65-1.18 volts), while the peaks reached by the seven infected dogs, together with the respective times after infection at which they occurred (in parenthesis), are: 2.61 cm. (144 hours), 2.20 cm. (127 hours), 2.14 cm. (92 hours), 2.13 cm. (93 hours), 2.11 cm. (144 hours), 2.00 cm. (46 hours), and 1.61 cm. (102 hours). The greatly increased wave height of the peak in the first-mentioned animal is very interesting, since the dog died during the night after this particular blood sample had been taken. Profound alterations in the serum of this dog must have occurred, and speculations as to the relationship between the changes in the serum, which are measurable by the polarograph, and to the cause of death, are very attractive and worthy of a continued study of this problem.

The observed decrease in the galvanometer deflection when whole serum is studied amounts to 10 to 15 per cent of the normal value. This change can undoubtedly be attributed to changes in the solid content of the serum, for a dilution of the serum of from 8 to 13 per cent is found in the case of two of the dogs studied. At least part of this decrease in solid content must be

attributed to nitrogenous constituents, for the total nitrogen values drop from 1,000-1,100 mg. per cent to 800-950 mg. per cent.

The nitrogen values of the peptone fraction, on the other hand, increase during infection by about 100 per cent and parallel closely, in time and percentage, the changes in the polarographic wave height. This can be seen in Fig. 4, curves I and II, where for better comparison the polarographic peptone wave height, as well as the milligrams of peptone nitrogen of Dog VI, are plotted against time after infection. Curve III in Fig. 4 represents degrees of fever during the infection. It is felt that while the nitrogen values agree fairly well with the changes in polarographic wave height, the same corollary with fever does not hold nearly so well. At least fever is not the direct cause of the observed polarographic changes, since the onset, rise, and fall in the fever curves of all seven dogs occur much sooner and more rapidly than the polarographic changes.

SUMMARY

Polarographic studies of normal and of type I pneumococci-infected dog sera are presented.

The wave height of the current-voltage curves of whole sera decrease during infection and return to normal with recovery. It is felt that these changes may be associated with corresponding shifts in the protein-cystine content of the sera.

The polarograms of the peptone fraction of dog sera, i.e., that fraction which, after alkali denaturation, is not precipitated by sulfosalicylic acid, increase in wave height as well as change their shape during infection. Recovery is associated with a return to normal values. With the increase in wave height during illness, a corresponding increase in nitrogen values occurs, while the fever curves do not directly parallel the polarographic changes.

We are greatly indebted to Mr. O. E. Sundberg, of the Calco Chemical Division of the American Cyanamid Co., for his cooperation in determining all the serum nitrogen values reported in this paper by the micro-Kjeldahl method.

REFERENCES

1. Brdicka, R.: Application of the Polarographic Effect of Proteins in Cancer Diagnosis, *Nature* 139: 330, 1937; Polarographic Investigations in Serological Cancer Diagnosis, *Nature* 139: 1020, 1937; Die Anwendung der polarographischen Methode Professor Heyrovsky's für die serologische Diagnostik des Krebses, *Revue des Gesundheitswesens* 20: 1, 1938; Serologische Untersuchungen mit Hilfe der polarographischen Methode und ihre Bedeutung für die Krebsdiagnostik, *Acta, Union internat. contre cancer* 3: 13, 1938; Recherches polarographiques sur les protéines, *J. de chim. phys.* 35: 89, 1938.
2. Waldschmidt-Leitz, E.: Über diagnostisch verwertbare Veränderungen im Serum bei Krebs, *Angewandte Chemie* 51: 324, 1938. Waldschmidt-Leitz, E., and Mayer, K.: Erfahrungen zur polarographischen Krebsdiagnose, *Ztschr. f. physiol. Chem.* 261: 1, 1939.
3. Terrell, E. E., Robertson, O. H., and Coggeshall, L. T.: Experimental Pneumococcus Lobar Pneumonia in the Dog. I. Method of Production and Course of the Disease, *J. Clin. Investigation* 12: 393, 1933.
4. Brdicka, R.: Polarographic Studies With the Dropping Mercury Cathode. XXXI. A New Test for Proteins in the Presence of Cobalt Salts in Ammoniacal Solutions of Ammonium Chloride, *Collect. Czechoslov. Chem. Communicat.* 5: 112, 1933.

STUDIES IN DYSTROPHIA MYOTONICA*

VI. RESULTS OF GLUCOSE TOLERANCE TESTS

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THE hypothesis has been advanced that the muscular changes in dystrophia myotonica are of endocrine origin; certainly many of the later symptoms of the disease suggest endocrine dysfunction. Defects of carbohydrate metabolism⁶ and storage⁸⁻¹⁰ have also been suggested as the cause of the weakness and atrophy of the muscles. A study of the glucose tolerance in these patients might furnish some information about the state of the endocrine system and carbohydrate metabolism; differences in capillary and venous blood glucose during glucose tolerance tests might give some clue to the utilization of glucose by the tissues; a variation in response to different doses of glucose might disclose borderline pathologic change. Accordingly, observations were made on both capillary and venous blood following the ingestion by each patient of two different doses of glucose.

Previous reports on the subject of fasting blood sugars and sugar tolerance tests in the muscular dystrophies and other severe muscular disorders are meager and often conflicting. In cases with *dystrophia myotonica* Sehranke and Full¹ reported no glycosuria following 150 Gm. of glucose (1 case); Brock and Kay,² a normal fasting glycemia and no glycosuria following 200 Gm. of glucose (1 case); Keschner and Finesilver,³ a prolonged hyperglycemia following breakfast (1 case); d'Antona,⁴ a fasting hypoglycemia (2 cases); a fasting hyperglycemia (1 case); a normal, a low prolonged, and a high prolonged glucose tolerance test (1 case each); Morgulis and Spencer,⁵ consistent zigzag tolerance curves with glycosuria in some instances. In cases of *muscular dystrophy*† Janney, Goodheart, and Isaacson⁶ found a lasting hypoglycemia (9 cases); McCrudden and Sargent,⁷ fasting hypoglycemia (3 cases, progressive type); Hughes and Shrivastava,⁸ hyperglycemia following 50 Gm. of glucose (2 cases, Erb's type); Braestrup,⁹ normal fasting glucose (3 cases); prolonged hyperglycemia and normal capillary-venous blood sugar differences following 1 Gm. of glucose per kilogram body weight (2 cases); normal glycemia and no capillary-venous differences (1 case). In cases with *myasthenia gravis* McCrudden and Sargent⁷ reported a slight fasting hypoglycemia (2 cases); Williams and Dyke,¹⁰ a normal tolerance test following 50 Gm. of glucose but a prolonged

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†In early, and even in more recent reports, dystrophia myotonica has not always been distinguished from other muscular dystrophies. From the descriptions given by Janney, Goodheart, and Isaacson, it appears quite likely that at least two of their patients may have had dystrophia myotonica. Likewise, the patient described by Brock and Kay undoubtedly had dystrophia myotonica rather than myotonia congenita.

hyperglycemia following 100 Gm. (3 cases); Adams, Power, and Boothby,¹¹ normal glycemia (5 cases). In one case with *progressive muscular atrophy* Braestrup⁹ found no capillary-venous differences during the glucose tolerance test.

METHOD

The 7 patients used in this study have been described in a previous article of this series.¹² The controls, in the same age groups as those with dystrophia myotonica, were 5 clinic patients and one of us (M. R.), carefully examined and found normal. None of the persons used in these studies was undergoing any kind of treatment at the time of the test or for a week previously.

The experiments were performed in the morning on persons who had received no food since the preceding evening. Blood was withdrawn from the brachial vein through a small needle into a syringe and immediately transferred from the tip of the syringe to two Folin¹³ blood pipettes calibrated "to contain"; the blood was discharged into two tubes of acid cadmium solution, and in the pipettes were thoroughly rinsed by drawing the solution up into them several times. Duplicate samples were then taken from a finger tip* directly into two Folin blood pipettes and discharged into the acid cadmium solution. An average of 3.4 minutes (range two to seven minutes) elapsed between the completion of the venous sampling and the completion of the capillary sampling. After a sample of urine had been collected, the patient received the measured amount of glucose dissolved in 250 c.c. of water flavored with lemon juice. Blood and urine samples were again taken at one-half, one, two, and three hours after the ingestion of glucose. All patients spent the time during the test in reading, resting, and walking about the halls. The test was performed twice within two weeks on each person, once with 50 Gm. of glucose and once with 100 Gm., though not necessarily in this order. Blood sugar values were determined by the Miller and Van Slyke¹⁴ method. All solutions were repeatedly rechecked or standardized as required; all volumetric apparatus was carefully calibrated at the beginning of the work. Duplicate analyses were performed in all the experiments and gave an average difference of 2.6 mg. per cent, with a range of 0 to 9 mg. per cent.

RESULTS

In all cases the fasting level of the blood was normal and no differences were evident between the controls and the patients in this respect. No glycosuria occurred in any of the tests. To conserve space, the individual data on the controls, which gave normal glucose tolerance curves in all respects at both levels of glucose, have merely been summarized in Table I. Since the rise in blood sugar is greater in the capillary blood than in the venous blood, two tolerance curves, one for the venous and one for the capillary blood, were obtained for each test, but the interpretation of the results of these tests is similar.

Results With 50 Gm. of Glucose.—The results for patients with 50 Gm. of glucose are very similar to those for the controls. The fasting values rise sharply

*Foster¹⁵ demonstrated that in studies on blood glucose, cutaneous blood could be substituted for pure arterial blood.

TABLE I
GLUCOSE TOLERANCE TESTS ON PATIENTS WITH DYSTROPHIA MYOTONICA

CASE	SEX	AGE	FASTING		30		60		120		180	
			CAP.	VEN.	CAP.	VEN.	CAP.	VEN.	CAP.	VEN.	CAP.	VEN.
J. B.	♂	60	79.2* 81.1	76.5 86.2	112.1 115.2	99.8 110.0	136.0 133.8	113.8 114.5	134.3 118.3	113.3 97.7	109.5 110.5	99.8 82.3
N. P.	♂	54	89.0 89.5	96.5 83.5	160.8 164.6	123.3 119.5	126.6 159.2	94.8 139.6	101.0 161.9	92.8 127.5	80.5 105.2	73.0 85.5
J. M.	♀	46	92.5 82.5	81.6 84.1	130.5 143.2	108.0 127.6	100.5 107.8	72.5 99.6	94.5 102.1	81.5 94.5	71.1 109.8	72.0 38.0
F. B.	♂	44	87.2 85.7	86.9 84.3	132.7 150.4	116.5 134.0	156.3 148.3	138.3 135.3	114.3 133.6	105.3 134.5	76.6 124.1	74.3 125.8
R. B.	♂	28	77.4 77.4	81.2 77.0	129.7 130.6	111.0 95.7	101.4 103.1	80.6 76.9	88.5 95.7	82.1 60.6	67.3 87.3	66.1 63.5
O. M.	♂	43	82.9 85.5	83.1 85.1	124.8 129.3	112.0 106.6	80.4 78.7	52.5 53.0	86.3 91.6	73.1 67.1	70.5 73.3	68.0 68.9
N. M.	♀	58	88.0 96.2	84.7 93.3	145.0 135.0	115.2 102.5	123.8 142.0	95.8 91.0	108.7 138.0	103.8 108.5	78.8 116.0	77.3 101.0
Patients, 50 Gm. avg.			85.2	84.4	133.7	112.3	117.9	92.6	103.9	93.1	79.2	75.8
Patients, 100 Gm. avg.			85.4	84.9	138.3	113.7	124.7	101.4	120.2	98.6	103.7	89.7
Controls, 50 Gm. avg.			87.1	87.3	148.6	126.4	140.4	115.9	91.7	82.7	77.0	76.0
Controls, 100 Gm. avg.			86.0	85.5	148.1	122.7	119.7	95.3	113.1	94.9	78.7	73.5

*Figures in roman type represent values obtained with 50 Gm. of glucose; those in italics with 100 Gm. of glucose. Blood values as milligrams per cent at minutes noted.

from normal to a peak, which is usually reached at thirty minutes but may be delayed until sixty minutes; the values then decline at the end of the third hour to below the fasting level, with one exception (J. B.). The peaks of the curves in both venous and capillary blood average about 15 mg. per cent less than the corresponding controls, but the variations from individual to individual in both patients and controls are so great that these differences scarcely can be considered significant. The peaks for the venous curves appear somewhat low, with a range of 108.0 to 138.3 and an average of 117.4 mg. per cent. However, since this represents an average increase of approximately 39 per cent over the fasting level, and since the smallest percentage increase is 26.7, these curves can be considered normal.

Comparison of the Results Obtained Following 100 Gm. of Glucose With Those Obtained Following 50 Gm.—MacLean and de Wesselow,¹⁶ and Williams and Dyke,¹⁰ found that in normal persons increasing the dose of sugar over about 25 to 50 Gm. gave no increase in the peak of the resulting hyperglycemia but tended to prolong the period during which the blood sugar was raised. Gray¹⁷ noted that the variations in glucose tolerance curves were as great from individual to individual as from different sized doses. Strouse¹⁸ reported that blood sugar values obtained in normal persons by giving 100 or 150 Gm. of glucose could be considerably increased in the same individuals by 200 Gm. of glucose, and Williams and Dyke¹⁰ found a difference in the glucose tolerance curves obtained with 50 and 100 Gm. of glucose in 3 patients with myasthenia gravis.

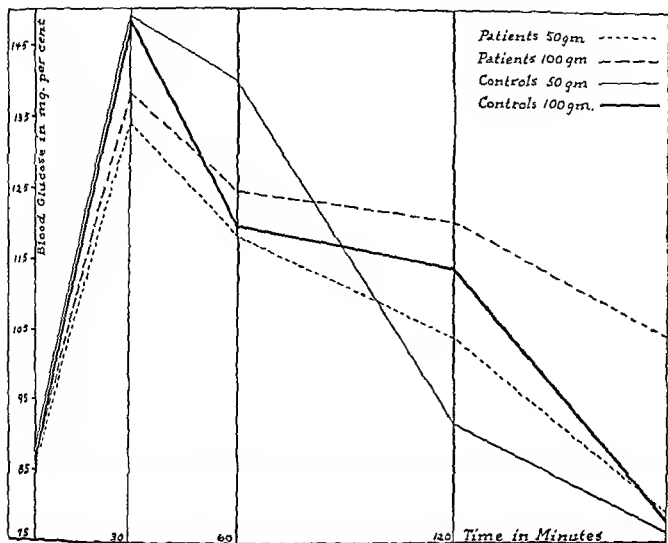


Chart 1.—Glucose tolerance curves on capillary blood.

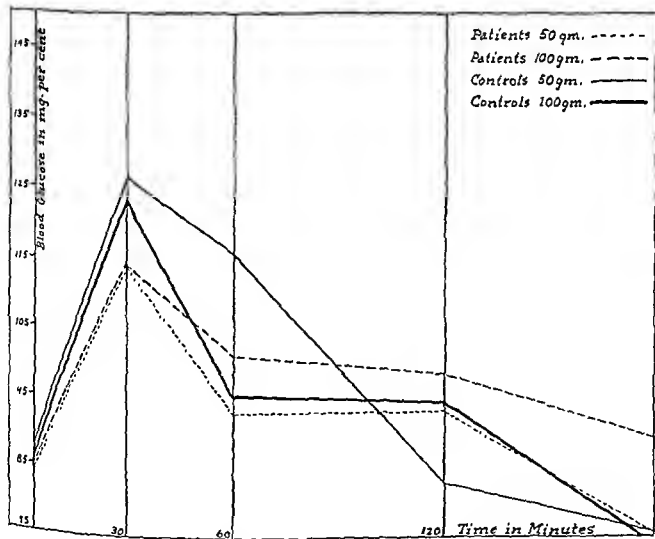


Chart 2.—Glucose tolerance curves on venous blood.

Although the individual curves of the controls receiving 100 Gm. of glucose show variations,* the composite curves are similar to those produced by 50 Gm. and give little evidence of a more prolonged hyperglycemia with 100 than with 50 Gm. of glucose.

The composite curves following 100 Gm. of glucose in patients tend to remain somewhat elevated during the second and third hours as compared with 50 Gm. (Table I and Charts 1 and 2). Thus, in the capillary bloods the final values with 100 Gm. average about 25 mg. per cent above the corresponding values with 50 Gm.; in the venous bloods, they average about 14 mg. per cent higher. This slight prolongation of the hyperglycemia with 100 Gm. is also evident when the final blood glucose values are compared with the initial values: at the 50 Gm. level only one patient had a higher final than fasting blood sugar (J. B. on venous and capillary blood); at the 100 Gm. level, 6 patients had higher final than fasting values (N. P., J. M., F. B., and N. M. on venous and capillary blood; J. B. and R. B. on capillary blood only). While the two levels of glucose gave somewhat different results in the patients than in the controls, the number of cases is so small and the variations from individual to individual are so marked that one cannot say whether these different results are merely chance or are indicative of a somewhat prolonged hyperglycemia at the higher glucose level.

Zigzag curves, usually less marked than those described by Morgulis and Spence² in patients with dystrophia myotonica, occurred among both controls and patients. Although somewhat more numerous in the patients than in the controls, their rather frequent occurrence in routine glucose tolerance tests indicates that these curves are of no special significance in dystrophia myotonica.

TABLE II
AVERAGE CAPILLARY-VENOUS BLOOD GLUCOSE DIFFERENCES

SUBJECTS, DOSE	FASTING	30	60	90	120
Controls, 50 Gm.	0.2*	22.2	24.5	9.0	1.0
Controls, 100 Gm.	0.5	25.4	24.4	18.2	5.2
Patients, 50 Gm.	0.8	21.4	25.3	10.8	3.4
Patients, 100 Gm.	-0.5	24.6	23.3	21.6	14.0

*Blood values as milligrams per cent at minutes noted.

Capillary-Venous Differences.—Our data for the capillary-venous blood sugar differences show practically identical results for both patients and controls after the ingestion of 50 Gm. of glucose (Table II). In both cases the differences are zero or very small at the fasting level; they rise to an average maximum of about 25, with a range of 18 to 31.8 mg. per cent during the first hour, as a rule, and then approach zero at the end of the third hour. These figures are almost an average for the arterial-venous differences reported in the literature (Foster;^{14, 19} Lundsgaard and Holbøll;²⁰ Rabinowitch;²¹ Frieden-son, Rosenbaum, Thalheimer, and Peters;²² and Cavett and Seljeskog²³). With 100 Gm. the capillary-venous differences decline to their original level somewhat more slowly, especially in the case of the patients (see Charts 1 and 2); how-

*In considering our data on the two levels of glucose, care must be taken to compare venous values with the corresponding venous values; the same is true of the capillary values; obviously the latter will be much higher throughout the hyperglycemia than the former.

ever, no statistically significant difference is evident between the controls and the patients receiving 100 Gm. of glucose. One control showed the maximum difference at the end of the second hour in both tests; one patient (J. B. at 100 Gm.), the most severely affected clinically, showed a progressively increasing difference through the third hour—a result of special interest, since no capillary-venous difference might have been expected if severe muscular atrophy were a factor in limiting this difference.

SUMMARY

Glucose tolerance tests were performed on 7 patients with dystrophia myotonica and on 6 controls. In each patient the test was performed once with 50 Gm. of glucose and once with 100 Gm., and in each test both capillary and venous blood were examined.

The fasting blood sugars and the glucose tolerance curves were normal in all patients; no glycosuria was evident. Composite glucose tolerance curves showed slight differences between patients and controls: (1) The glucose tolerance curves were slightly flatter in the patients than in the controls (2) The composite curves for 100 Gm. tended to remain elevated in the patients at the second and third hours, especially in the capillary blood. (3) The capillary-venous differences remained slightly higher in patients than in controls receiving 100 Gm. of glucose. None of these differences was great enough to be statistically significant, and no correlation was found between variations in response to the tests and the severity of the disease.

Since marked dysfunction of the islands of Langerhans, the anterior pituitary gland, or the adrenal cortex is usually reflected in the fasting blood sugar and in the sugar tolerance curve, the results obtained furnish some evidence against a marked involvement of these endocrine glands. The removal of glucose from the capillary blood by the tissues, as indicated by the capillary-venous differences, also appears normal. It may be concluded that the mechanisms controlling the disposal of ingested glucose in dystrophia myotonica are normal.

We wish to express our appreciation to Dr. James J. Waring for pointing out the value of the glucose tolerance test in dystrophia myotonica and to him and Dr. Robert C. Lewis for their many helpful suggestions during the progress of this work and the preparation of this manuscript.

REFERENCES

1. Schranke, and Full: Innere Sekretion und Myotonische Dystrophie, *Ztschr. f. d. ges. Neurol. u. Psychiat.* 61: 146, 1920.
2. Brock, S., and Kay, W. E.: A Study of Unusual Endocrine Disturbances; Their Associated Myopathies, Endocrine Balance and Metabolism Findings, *Arch. Int. Med.* 27: 1, 1921.
3. Keschner, M., and Finesilver, B.: Myotonia Atrophica (Dystrophia Myotonica), *J. Neurol. & Psychopath.* 5: 341, 1925.
4. d'Antona, L.: Osservazioni sullo stato dell' apparato circolatorio e digerente nella distrofia miotonica. La sindrome endocrina ed Umorale, *Minerva med.* 1: 833, 1935.
5. Morgulis, S., and Spencer, H. C.: Studies on the Blood and Tissues in Nutritional Muscular Dystrophy, *J. Nutrition* 12: 173, 1936.
6. Janney, N. W., Goodheart, S. P., and Isaacson, V. L.: The Endocrine Origin of Muscular Dystrophy, *Arch. Int. Med.* 21: 188, 1918.
7. McCrudden, F. H., and Sargent, C. S.: Chemical Changes in the Blood and Urine in Progressive Muscular Dystrophy, Progressive Muscular Atrophy and Myasthenia Gravis, *Arch. Int. Med.* 21: 252, 1918.
8. Hughes, T. A., and Shrivastava, D. L.: Carbohydrate Tolerance in Two Cases of Muscular Dystrophy, *Indian J. M. Research* 15: 437, 1927.

9. Bracstrup, P. W.: The Action of Glycine (Glycocol) on Muscular Dystrophy and Other Diseases, *Acta med. Scandinav.* 89: 231, 1936.
10. Williams, B. W., and Dyke, S. C.: Observations on Creatinuria and Glycosuria in Myasthenia Gravis, *Quart. J. Med.* 15: 269, 1921-22.
11. Adams, M., Power, M. H., and Boothby, W. M.: Chemical Studies in Myasthenia Gravis, *Ann. Int. Med.* 9: 823, 1936.
12. Waring, J. J., Ravin, A., and Walker, C. E.: Studies in Dystrophia Myotonica. II. Clinical Features and Treatment, *Arch. Int. Med.* 65: 763, 1940.
13. Folin, O.: A New Blood Sugar Method, *J. Biol. Chem.* 77: 421, 1928.
14. Foster, G. L.: Some Comparisons of Blood Sugar Concentrations in Venous Blood and in Finger Blood, *J. Biol. Chem.* 55: 291, 1923.
15. Miller, B. F., and Van Slyke, D. D.: A Direct Microtitration Method for Blood Sugar, *J. Biol. Chem.* 114: 583, 1936.
16. MacLean, H., and de Wesselow, O. L. V.: The Estimation of Sugar Tolerance, *Quart. J. Med.* 14: 103, 1921.
17. Gray, H.: Blood Sugar Standards. Part I. Normal and Diabetic Persons, *Arch. Int. Med.* 31: 241, 1923.
18. Strouse, S.: Observations on Alimentary Hyperglycemia, *Arch. Int. Med.* 26: 759, 1920.
19. Foster, G. L.: An Interpretation of the Blood Sugar Phenomena Following the Ingestion of Glucose, *J. Biol. Chem.* 55: 303, 1923.
20. Lundsgaard, C., and Holbøll, S. A.: Studies in Carbohydrate Metabolism. III. Investigations Into the Nature of the Glucose in the Blood of Normal Individuals, *J. Biol. Chem.* 65: 323, 1925.
21. Rabinowitch, I. M.: Simultaneous Determinations of Arterial and Venous Blood-Sugars in Diabetic Individuals, *Brit. J. Exper. Path.* 8: 76, 1927.
22. Friedenson, M., Rosenbaum, M. K., Thalheimer, E. J., and Peters, J. P.: Cutaneous Venous Blood Sugar Curves. I. In Normal Individuals After Insulin and in Liver Disease, *J. Biol. Chem.* 80: 269, 1928.
23. Cavett, J. W., and Seljeskog, S. R.: Comparison of Sugar Tolerance Curves Obtained on Venous and Capillary Blood, *J. LAB. & CLIN. MED.* 18: 1103, 1933.

LABORATORY METHODS

GENERAL

A VESSEL FOR THE MEASUREMENT OF pH AND OXIDATION-REDUCTION POTENTIAL OF BACTERIAL CULTURES*

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THE determination of the changes of the pH and the oxidation-reduction potential which a culture of bacteria undergoes during the course of its metabolism is of considerable interest to workers concerned with the problem of the physiology of bacteria. The difficulty of following these changes over more or less lengthy periods of time without the danger of contamination has interested us in designing the culture vessel described below.

The details of construction are evident in Fig. 1, and consist essentially of three electrodes, calomel, glass, and platinum, sealed in a No. 40 standard taper, pyrex inside joint by means of red sealing wax. These electrodes dip into the bacterial culture contained in the vessel constructed from the outside member of a No. 40 standard taper ground joint. By appropriate means the pH may be obtained from the voltage difference between the glass and calomel electrodes. The oxidation-reduction potential may be measured by using the calomel and platinum electrodes; or under special conditions, by the glass and platinum electrodes described by Wynd.³

Electrode 1 is a saturated potassium chloride: calomel half cell. The side arm *G* passes through the base of the standard joint, as indicated, and dips into the culture. The contact *H* is made through a small piece of porcelain bacteriologic filter candle sealed into the end of the tube *G*.

Electrode 2 is a double shank glass electrode constructed as described by Varney.² The bulb is filled with 0.1 N hydrochloric acid and contains a silver: silver chloride electrode whose external contact is indicated by the numeral 2 in the figure. The silver wire passes through a small glass tube of about 3 mm. outside diameter, which is sealed into the inner tube *B* of the glass electrode by red sealing wax. *C* is the outer shank of the glass electrode unit by means of which a very high degree of electrical insulation is obtained.

Electrode 3 furnishes the platinum surface *D*, whose simple construction requires no comment.

The culture vessel itself contains about 50 c.c. of bacterial suspension. The side arm *O* may be closed with a cotton plug, or a steel ball bearing may be inserted to permit anaerobic conditions and allow for the escape of carbon

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dioxide. It is necessary first to clear the culture vessel of oxygen by passing nitrogen through the apparatus by means of the opening in the plug of stopcock *N*. The degree of anaerobiosis obtained is sufficient for many types of bacteria. Side arm *P* and stopcock *N*, are so constructed as to permit the removal of uncontaminated drops of the culture at intervals without disturbing the electrode arrangement. Various types of side arms may be constructed for the practical manipulation of various types of experiments.

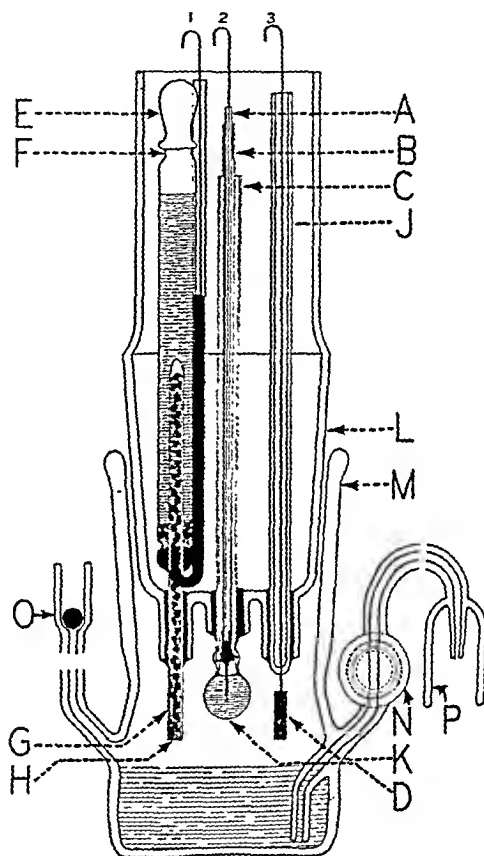


Fig. 1.—Culture vessel and arrangement of the electrodes. 1, Contact of the calomel half cell; 2, contact of the glass electrode; 3, contact of the platinum electrode; A, capillary tube filled with red wax and carrying the silver wire into the bulb of the glass electrode; B, the inner shank of the glass electrode which seals the outer shank C at the base; D, bulb of the glass electrode containing 0.1 N hydrochloric acid; E, rubber bulb closing the calomel half cell; F, the calomel half cell; G, side arm of the calomel half cell filled with saturated potassium chloride containing crystals of potassium chloride; H, piece of porcelain filter candle sealed into the glass side arm of the calomel half cell; I, shank of the platinum electrode; K, platinum foil; L, inside member of a No. 40 standard taper ground joint; M, outside member of the ground joint; N, ungreased stopcock; O, ball bearing or cotton plug; P, cup protecting the enclosed tip from contamination.

The technique of operation is simple but must be carefully done. The vessel *M* and its side arm *O* are plugged with cotton and autoclaved. The structure *L* is sterilized by dipping it into a solution of calcium hypochlorite for a few seconds, washed with sterile water, and placed in sterile water in a duplicate vessel *M* until ready for use. It is advisable to permit the electrodes to stand in the sterile water overnight in an incubator at 37° C. to insure the complete removal of the calcium hypochlorite from the electrically active surfaces. The culture is next added to the vessel, and the electrodes are quickly inserted.

The series of culture vessels is then connected to the proper contacts by hooking the wires 1, 2, 3, shown in Fig. 1 over the spring brass wires shown in Fig. 2. This device is constructed on a bakelite base and the electrical contacts are screwed to the upper ends of six inch polystyrene insulators. (The wiring is arranged as shown in Fig. 3.) The calomel contact 1, in Fig. 1, hooks over the spring wire *P*, the glass electrode contact 2, in Fig. 1, hooks over wire *G*, and the platinum electrode contact 3, in Fig. 1, connects to the spring wire *C*.

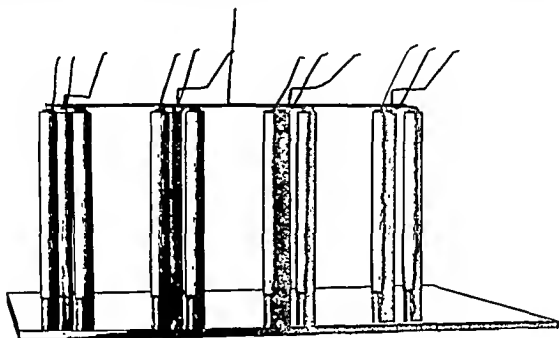


Fig. 2.—Series of electrode contacts within the incubator.

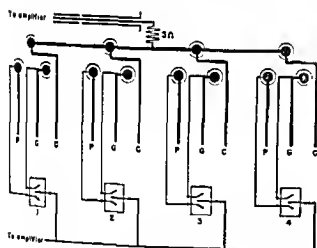


Fig. 3.

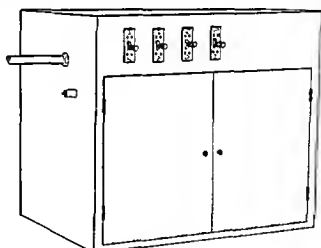


Fig. 4.

Fig. 3.—Wiring diagram of the electrode platform shown in Fig. 2

Fig. 4.—Incubator chamber, showing the arrangement of the control switches and the leads to the amplifier.

The complete electrode platform rests on porcelain insulators inside the incubator shown in Fig. 4. This incubator chamber is constructed of sheet iron or duralumin. The Federal Switches shown in Fig. 4 are those indicated as 1, 2, 3, 4, in Fig. 3. The common lead from the four calomel electrodes is attached to the control grid of a vacuum tube amplifier by passing through a pyrex tube, as shown on the end of the incubator. The common lead from the central contact of the four Federal Switches, as shown in Fig. 3, is brought to the proper terminal of the amplifier by means of the porcelain insulator shown on the end of the incubator.

The current from the various pairs of electrodes is amplified by the usual vacuum tube apparatus, and the voltage is read from a potentiometer. The type of amplifier described by Skow and Wynd,¹ and by Wynd and Varney,⁴ functions very satisfactorily when used in this manner.

Examination of Figs. 3 and 4 shows that the pH, or the oxidation-reduction potential of any of the four cultures may be obtained conveniently by the proper manipulation of the Federal Switches and by the observation of the corresponding voltage. By means of this apparatus we have successfully followed the changes in pH and the oxidation-reduction potential of bacterial cultures over periods as long as six days.

SUMMARY

1. The pH and the oxidation-reduction potential of a series of bacterial cultures may be observed over comparatively long periods, without disturbing the cultures, by means of a series of vessels within a metal incubator, each containing a glass, platinum, and calomel electrode, properly connected to a vacuum tube amplifier through the wall of the incubator.

2. Observations have been obtained successfully over periods as long as six days without removing the cultures from the incubator.

REFERENCES

1. Skow, Royce K., and Wynd, F. Lyle: An Apparatus for the Amplification of Glass Electrode Potentials, *J. LAB. & CLIN. MED.* 22: 316, 1936.
2. Varney, Philip L.: Glass Electrodes, *Science* n. s. 82: 396, 1935.
3. Wynd, F. Lyle: A Note on the Application of the Glass Electrode to the Determination of Oxidation-Reduction Potentials, *Ann. Missouri Bot. Gard.* 22: 861, 1935.
4. Wynd, F. Lyle, and Varney, Philip L.: A Direct Reading Null Point Amplifier for Measuring Hydrogen Ion Concentrations and Oxidation-Reduction Potentials, *J. LAB. & CLIN. MED.* 23: 864, 1938.

A SIMPLIFIED WESTERGREN SEDIMENTATION RATE TECHNIQUE

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THE Westergren sedimentation tube has been modified by various people to facilitate the technique of the test. All of the modifications, however, require extra apparatus, such as stopcocks, racks, or special pipettes. We have been using a technique that in our opinion is simpler, requires no additional parts, except a rubber diaphragm, and is accurate.

TECHNIQUE

The diaphragm used is simply the rubber bulb of a small dropper, such as the one supplied with sample nose drops. The end of the rubber bulb is cut off and the base is placed over the bottom of the Westergren tube. The blood is drawn from the vein in the usual manner, and the proper amount of anticoagulant is then drawn into the same syringe. Then after introducing some air, the blood and anticoagulant are mixed by gentle agitation of the syringe. The

mixture is then introduced into the Westergren tube by inserting the needle through the rubber diaphragm. By using this technique the level of the blood in the tube can be carefully regulated. On withdrawal of the needle the rubber diaphragm seals itself off. With this procedure the intermediate steps between

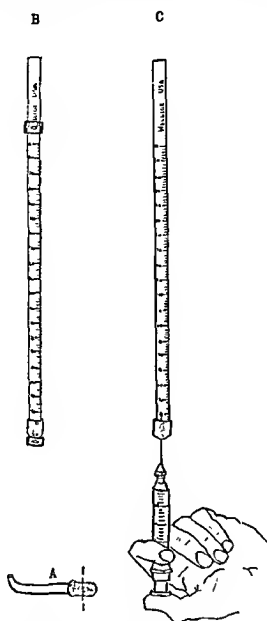


Fig. 1—A, Sample dropper showing line of cut. B, Tube held on wall with staples. C, Technique of injecting blood into tube

taking the blood and its introduction into the tube are simplified. The tube is then held on the wall by two staples. The tube rests on the lower staple while the upper one assures the exact vertical position of the tube. The advantage of hanging the tube on the wall in this manner is its simplicity and its safety from any agitation which would invalidate the test. This technique is simple in procedure, costs nothing, and is accurate.

A MODIFICATION OF THE GRAM STAIN*

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NUMEROUS modifications of the Gram stain have been recommended since the publication of the original method.¹ These attempts can be appreciated for the following reasons: (1) The Gram stain is an important and frequently used procedure; (2) the results vary when the same method but different ingredients are used; (3) the outcome depends to some extent upon the skill and experience of the workers. Some of the more recently used modifications^{2, 3} proposed a far more complicated technique in an effort to overcome some previous complaints and handicaps. However, they still retained a few of the previous disadvantages, especially the instability of the ingredients.

I thought it worth while to utilize my experiences with the Gram stain and to devise a procedure which would be more accessible to the average laboratory worker for the daily routine.

Generally one of the most frequently observed difficulties for the ordinary worker represents the time factor of decolorization. Ninety-five per cent alcohol decolorizes too slowly, and acetone decolorizes too rapidly. Probably the next complication in importance for the less experienced worker consists of over-staining with the counterstain. This difficulty can be more easily overcome by using weaker staining solutions for a shorter period of time. The next important obstacle consists of the staining of smears which are not uniformly and usually too thickly spread. In order to decolorize the thicker portions sufficiently, the thinner parts are too extensively decolorized, thus sometimes confusing a proper interpretation of the findings. A too concentrated solution of gentian violet may not keep and might form a sediment; a too weak solution may not produce the desired results. The same observation applies to similar dyes which are occasionally used instead of gentian violet. The ordinary Gram's iodine gives quite different results, depending upon whether the gentian violet: (1) was just poured off, (2) was washed off with water, (3) was washed off with Gram's iodine, or (4) was washed off and dried preceding the application of Gram's iodine. Apparently a prolonged application of gentian violet and Gram's iodine in excess of three minutes has very little additional effect on the results.

Our own experimental work considered the following factors: (1) concentration and stability of the used ingredients; (2) temperature of the solutions at the time of application; (3) application of additional solutions preceding the use of Gram's iodine in order to intensify the staining of gram-positive bacteria; (4) selection of a satisfactory decolorizant; (5) most effective counterstain.

It was found that a 3 per cent solution of gentian violet in a 20 per cent alcoholic solution (gentian violet 3 Gm., 95 per cent alcohol 20 c.c., distilled

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water 80 c.c.) keeps well. Similarly, a twenty times increased concentration of Gram's iodine (20 Gm. of iodine, 40 Gm. of potassium iodine, and 300 c.c. of distilled water) also keeps well and has an increased effect over the ordinary Gram's iodine. Fuchsin was preferred to safranin on account of its bright color. A 2 per cent solution of basic fuchsin in 95 per cent alcohol was found satisfactory, stable, and preferable to other dyes. This stain should be diluted 1:4 with distilled water previous to its use. After considerable experimentation acetone was found to be the most effective decolorizant. Steaming with gentian violet for one minute and subsequent steaming with concentrated Gram's iodine for the same length of time was found slightly preferable to the same procedure without steaming. A series of 10 per cent salt solutions were tried on the smears following the washing off of the gentian violet. A freshly prepared 10 per cent solution of sodium carbonate was found to be very satisfactory; however, the advantage was not sufficiently great to include this step in the proposed procedure.

Consequently the following modification of the Gram stain has been devised:

- (1) Prepare smears as thinly and uniformly as possible and fix them over the flame.
- (2) Cover the slide with 3 per cent gentian violet for three to five minutes.
- (3) Wash with warm water.
- (4) Cover with twenty times concentrated Gram's iodine for three to five minutes.
- (5) Wash with warm water.
- (6) Cover (decolorize) the slide with acetone and wash off immediately with water.
- (7) Counterstain briefly (on and off) with above dilution of fuchsin.
- (8) Wash with water, allow to dry, and examine under oil immersion.

DISCUSSION

The proposed modification compares favorably with a number of recently recommended procedures. All the reagents are easily prepared, keep for an indefinite period, and are applied without difficulty. Repeated filtrations and waste of the deteriorated reagents are avoided. Mixing of reagents or other technical steps preceding each individual staining are not required in this technique. The technique of preparing or applying the reagents does not require any particular skill. The differentiation between gram-positive and gram-negative bacteria is very marked. Smears from tissue discharges and those from cultures give equally good results. No sediment is found in the stained preparations. This procedure has been tried out with satisfactory results on a large number of bacteria. The tested bacteria included, among others, various pathogenic and nonpathogenic cocci, intestinal bacteria, diphtheria bacilli, and several anaerobes.

SUMMARY

The proposed modification of the Gram stain offers a number of definite advantages: (1) All reagents keep well. (2) The technique is simple and is easily carried out. (3) The distinction between gram-positive and gram-negative bacteria is very distinct.

REFERENCES

1. Gram, C.: Über die isolierte Färbung der Schizomyceten in Schnittpräparaten, Fortschr. d. Med. 2: 185, 1884.
2. Huecker, G. J., and Conn, H. J.: Modifications of the Gram Stain, Technol. Bull. 128, New York Agricultural Experimental Station, Geneva, N. Y., 1927.
3. Kopeloff, N., and Beerman, P.: Modified Gram Stains, J. Infect. Dis. 31: 480, 1922.

A GLASS-CAPSULE MANOMETER FOR RECORDING THE BLOOD PRESSURE*

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VARIOUS methods of recording the blood pressure continuously in acute experiments in mammals have included the use of glass membranes as in the apparatus of Broemser.¹ The apparatus to be described here has certain advantages, among the more important of which are its sturdiness and sensitivity without the objectionable inertia of the mercury manometer.

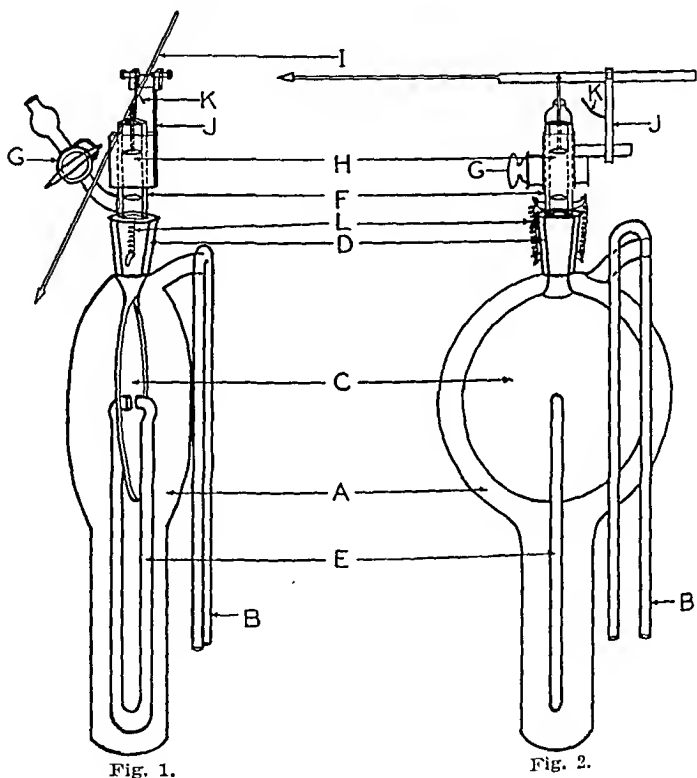


Fig. 1.

Fig. 2.

DESCRIPTION OF APPARATUS

Except for the writing lever and its supports (*I*, *J*, and *K*, Figs. 1 and 2) and the springs, *L*, the apparatus is made entirely of pyrex glass. The heavy-walled chamber *A* is attached to side limbs *B*, so that bubbles may be easily removed. It has been our practice to connect one limb of *B* to a modified Trendelenburg² mercury-sodium carbonate pressure system and the other limb to the arterial cannula. The whole system, including chamber *A*, is filled with

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0.1 M sodium carbonate. Capsule *C*, enclosed in chamber *A*, opens into the ground joint *D* and is supported by the U-shaped rod *E*. The glass of the capsule is 0.5 to 0.75 mm. thick. The recording device consists of cylinder *F*, fitting into the ground joint *D*, the attached stopcock *G*, piston *H*, lever *I*, support *J*, and lever stop *K*. Springs *L* firmly hold the recording device in place.

Capsule *C*, the capacity of which is about 7 c.c., is filled with distilled water. There must be no bubbles of air either in capsule *C* or in chamber *A*. If the apparatus is used under conditions causing rapid evaporation of water, a few drops of watchmaker's oil may be placed on top of the piston. Chamber *A* is filled with 0.1 M sodium carbonate (or other anticoagulant) and connected with the arterial cannula and the Trendelenburg apparatus. Stopcock *G* is opened and the writing lever is pressed down to lever stop *K*. This is the base line. The stopcock is now closed, the stopper is placed in the arterial cannula, and the artery clamp is removed. Ordinarily we have adjusted the apparatus so that a movement of 5 mm. corresponds to a pressure change of approximately 10 mm. of mercury. Exact calibration is, of course, done and accurately reproducible.

The error caused by changes in temperature is small; at approximately room temperature, each change of 1° C. is followed by a pressure change corresponding to 0.8 mm. of mercury.

REFERENCES

1. Broemser, P.: Das Hebelplattenmanometer, *Ztschr. f. Biol.* 85: 131, 1926.
2. Trendelenburg, P.: Verfahren zur Verhinderung der Blutgerinnung bei der Blutdruckschreibung, *Arch. f. d. ges. Physiol.* 203: 413, 1924.

A CLOSED-CIRCUIT METABOLISM APPARATUS FOR STUDYING OXYGEN CONSUMPTION OF CONTROL AND THYREOACTIVATOR-TREATED GUINEA PIGS*

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A METHOD that is both simple and rapid was devised to study oxygen consumption in small laboratory animals. The entire outfit can be assembled from apparatus available in most laboratories, thus making it easy to acquire and maintain as many sets as required.

A description of the apparatus and reports of oxygen consumption in control and treated guinea pigs follows:

The metabolism unit, as illustrated in Fig. 1, consists of four fundamental parts: a Mariotte or aspirator flask *a*, a graduated cylinder *c*, an animal chamber *i*, and a weighted stand *l*. With the animal resting in the hermetically sealed chamber on the wire mesh platform *j*, and with appropriate measures taken for the absorption of carbon dioxide by the soda lime *k*, the entire unit is submerged in a constant temperature water bath to the level of the neck of the Mariotte flask. At this time the clamp *c* is closed. In order to allow the ap-

*From the Department of Anatomy, University of Maryland Medical School. The work reported was aided by the Weaver Fellowship Fund.

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paratus to come to the temperature of the bath, an hour is allowed to elapse before readings are started. During this interval a constant current of air is passed through the system by connecting outlet *h* to a suction pump, with clamp *f* open. The opening of the side arm of the graduated cylinder to which clamp *f* is attached must be extended above the surface of the water by means of tubing. It was found advantageous to bubble the air through some water in cylinder *e*, so that the rate of flow could be estimated. The animals were found to rest very quietly throughout this preliminary period using this method.

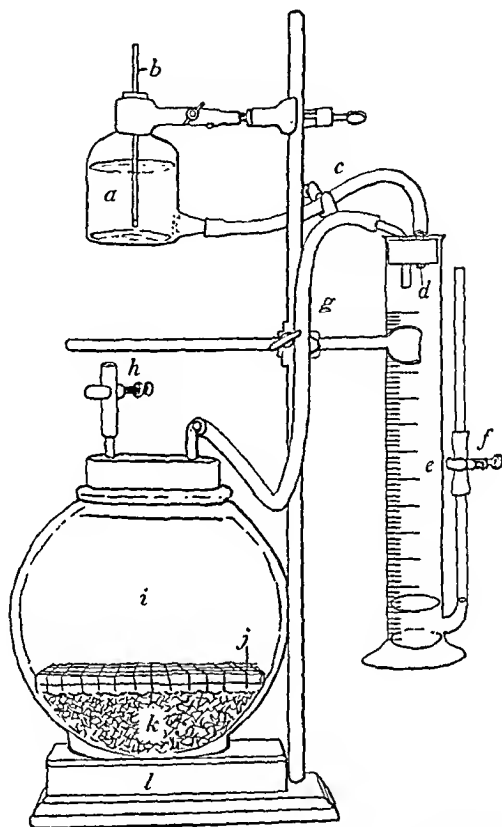


Fig. 1.—Showing the closed-circuit oxygen consumption apparatus, *a*, Mariotte flask; *b*, glass tube; *c*, rubber connection between flask and cylinder; *d*, opening of connection *c*; *e*, graduated cylinder, 200 to 250 c.c.; *f*, side arm on cylinder; *g*, rubber connection between cylinder and animal chamber; *h*, outlet of animal chamber; *i*, animal chamber; *j*, wire mesh platform for animal; *k*, soda line; *l*, weighted stand to keep apparatus submerged.

Before describing the procedure employed in taking a reading, the application of the Mariotte flask *a* deserves comment. This flask must be adjusted at a height that permits the water in the flask to rise in tube *b* about $\frac{1}{4}$ inch. The water will not flow from the Mariotte flask into the graduated cylinder until the pressure in the cylinder is decreased by the removal of oxygen from the system by the animal within the chamber. With the submerged outlet of tube *b* kept at a constant level, the pressure exerted by the receding height of the water in the Mariotte flask as it flows into the cylinder is kept constant as long as the water level in the Mariotte flask is above the opening of tube *b*. The Mariotte principle functions in such a manner that all the water above the level of the submerged opening of tube *b* serves merely as a reservoir, while the height of

the column of water that exerts pressure in the flowing system is determined by the level at which the atmosphere comes into contact with the water, i.e., at the level of the submerged opening of tube *b*. This avoids the variations that would otherwise be introduced by the gradually decreasing pressure of a diminishing column of water as it flows into the cylinder from the flask.

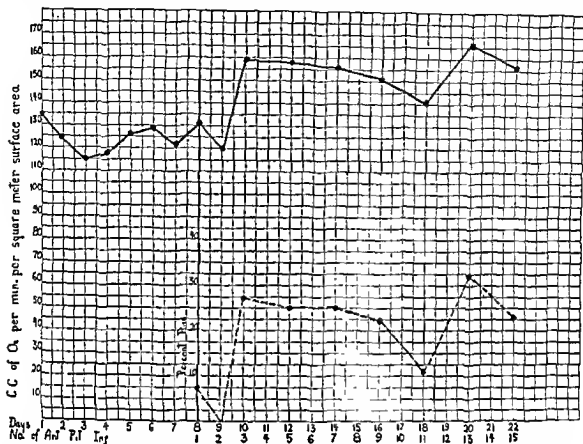


Fig. 2.—Graph showing the effect of daily doses of thyroactivator hormone derived from 25 mg. of dried beef anterior lobe powder on the oxygen consumption of a guinea pig

When ready to start a reading, the air or oxygen stream is stopped. Clamps *f* and *h* are closed, and clamp *c* is opened. When the animal has exhausted enough oxygen to lower the pressure inside the system, the water begins to flow from the Mariotte flask into the cylinder. After the flow has started, the height of the water in the cylinder is recorded and readings thereafter are made at any desired interval. The amount of water that flows into the cylinder is equivalent to the volume of oxygen consumed by the animal.

When the experiment is completed, clamp *c* is closed again, and clamps *f* and *h* are opened. The water is evacuated from the cylinder by applying suction to the side arm *f*, while tube *h* is open to the atmosphere. The water in the Mariotte flask is replenished from the water bath, and the oxygen supply is restored by a current of air as described. The readings can thus be repeated any number of times.

Theoretically barometric pressure corrections should be made for each reading. However, the barometric pressure changes are so slight in the case of readings of such short duration that they were found to be of very little significance in an apparatus of such large volume as that used for the guinea pigs. In studies of the oxygen consumption of salamanders using the principle described, with appropriate modifications to suit the size of the animal, Thompson and Uhlenhuth (1936) found that barometric pressure corrections were essen-

tial. Because of the small quantities of oxygen consumed, it was found desirable to take readings over six- to eight-hour periods, during which time the barometric pressure could change significantly.

An example of the results obtained with the apparatus described is shown in Fig. 2. Here the effect of daily injections of an extract derived from 25 mg. of dried anterior lobe powder (beef) on the oxygen consumption of a guinea pig is represented.

TABLE I

OXYGEN CONSUMPTION OF 15 YOUNG GUINEA PIGS

(Each value is an average of at least five readings taken on separate days.)

WEIGHT, GM.	162	145	156	132	137	160	159	146	172	150	150	172	115	147	167
SEX	M	M	M	M	M	F	F	F	F	F	F	F	F	F	F
C.C. OXYGEN SQ.M. PER MIN.	123	139	138	131	137	146	129	141	139	130	135	135	134	139	139

Table I gives records of 15 untreated guinea pigs of both sexes ranging from 115 to 172 Gm. in weight. The oxygen values are recorded in cubic centimeters per square meter body surface per minute. The experiments were done in a constant temperature bath at 31° C. Friedgood (1934), using a modified Benedict closed circuit apparatus at 32° C., obtained average oxygen values in cubic centimeters per square meter per minute of 105 c.c. for male and 99 c.c. for females. His guinea pigs, however, weighed between 400 and 650 Gm. The higher values revealed in Table I are undoubtedly due to the younger age of the animals. DuBois (1927), discussing the effect of age on metabolism, pointed out the inverse ratio existing between the two during the early periods of rapid growth.

SUMMARY

1. A closed-circuit apparatus for the study of oxygen consumption in small laboratory animals has been described. This apparatus has the advantages of simplicity and rapidity. It has been used satisfactorily with the guinea pig, rat (Krantz and others), and salamander.

2. Oxygen consumption of a guinea pig treated with thyreoactivator hormone is shown to be increased (Fig. 2).

3. Oxygen consumption of a colony of 15 young guinea pigs is shown to be comparable. No certain sex variations were observed (Table I).

REFERENCES

- DuBois, E. F.: *Basal Metabolism in Health and Disease*, ed. 2, Philadelphia, 1927, Lea & Febiger.
- Friedgood, H. B.: *Experimental Exophthalmos and Hyperthyroidism in Guinea-pigs*, Bull. Johns Hopkins Hosp. 54: 48, 1934.
- Krantz, J. C., Carr, C. J., and Beck, F. F.: *A Further Study of Barbiturate-Picrotoxin Antagonism*, J. Pharmacol. & Exper. Therap. 61: 153, 1937.
- Thompson, J. U., and Uhlenhuth, E.: *Effect of Prolonged Thyreoactivator Injections Upon Metabolism of Adult Salamanders*, Anat. Rec. 64: supp. p. 50, 1936.

A MODIFIED TUBE FOR TATTOO MACHINES*

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TATTOO has been employed in medical practice for the correction of cosmetic defects of the skin¹ and eye, and for the marking of laboratory animals. I introduced tattoo with mercuric sulfide for its therapeutic effect in pruritus ani.^{2, 3}

In tattoo an insoluble chemical is deposited through the epidermis into the corium by means of one or more needles. The standard machine consists of an armature (*L*) which vibrates through the magnetic action of two electromagnetic coils (*M*). A needle bar (*N*) attached to the armature passes through the tube (*B*). Six to 8 needles connected to the distal end of the bar move forward and backward with a thrust of 2 mm. by the vibration of the armature. The tube is held and moved like a writing pencil, with sufficient pressure to insure the penetration of the needles into the corium without lacerating the skin.

The purpose of the treatment of pruritus ani by tattoo is to deposit mercuric sulfide uniformly throughout the corium of the involved skin. When this is done, a uniform deep red tattoo results; the itching is relieved and the skin returns to its normal texture. To obtain a uniform tattoo, I use four upstrokes with each application of the chemical. The tube is held at an angle of 30 to 45 degrees to the skin. The corium is penetrated when the needles are kept in close contact with the skin and when sufficient current is used to make the armature vibrate freely against the resistance of the skin. It is essential that the chemical be always present at the needle points, and that these points lie parallel to the surface of the skin.

The following tube has been devised to insure the presence of a chemical at the needle points of a tattoo machine and to reduce the tension on the finger that directs the needles. It consists of a piece of hollow brass (*A*) $\frac{1}{4}$ inch in diameter and $1\frac{1}{4}$ inches in length. It is split lengthwise on its upper surface and is soldered to the tube (*B*) $\frac{1}{4}$ inch from its tip.

The distal end of the brass piece (*C*) is flattened to equal the width of the needles, and projects $\frac{1}{8}$ inch forward from the soldered base. When the tube is dipped into the chemical used for tattooing, this end acts as a reservoir to feed the needles. It is then practicable to increase the number of needles to the limit of the diameter of the tube, which is 18 needles. By increasing the number of needles from the customary 6 to 8, the time required to tattoo a certain area of skin is reduced.

Where the skin is thin, it is practicable to use 18 needles to produce a uniform deposit of the chemical throughout the corium, resulting in a uniform

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deep red tattoo. However, where the skin is hyperkeratotic or leathery, not more than 8 needles should be used. I have found that the resistance of such epidermis is too great for the uniform penetration of more than 8 needles.

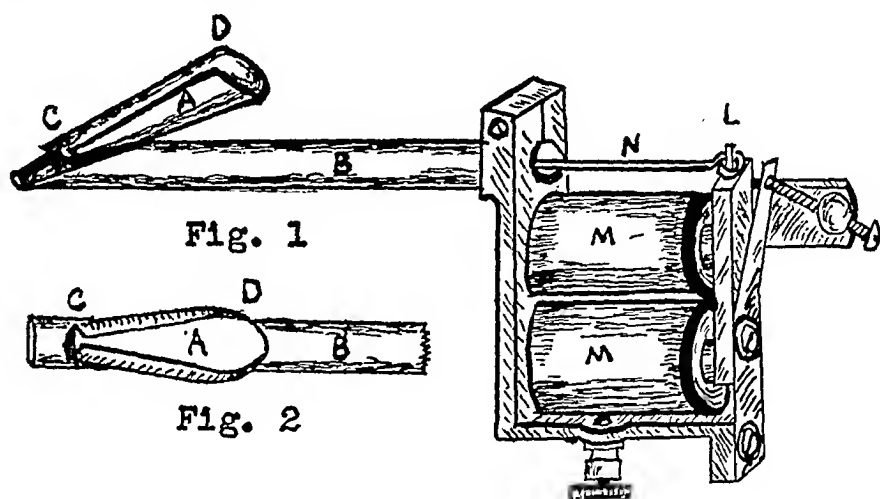


Fig. 1.—View of the tube and machine from the side and front.

Fig. 2.—View of the tube from the top and front.

The proximal end of the attachment (D) is opened into a smooth flange on which the thumb or index finger rests. This acts as a fulcrum to balance the weight of the machine (8 ounces) which reduces the pressure that the finger must exert to direct the needles on the skin. Preventing fatigue of the fingers is especially important in tattooing an irregular surface, such as the perianal region, for the direction of the needles must be changed frequently to keep their points parallel to the plane of the skin.

REFERENCES

1. Filips, L.: Artistic Tattooing in Dermatology, Arch. Dermat. & Syph. 22: 507, 1930.
2. Hollander, E.: Treatment of Pruritus Ani by Tattoo With Mercuric Sulfide, Arch. Dermat. & Syph. 38: 337, 1938.
3. Sulzberger, M. B.: The Effect of Tattooing With Mercury Sulfide on Pruritus Ani, Arch. Dermat. & Syph. 40: 493, 1939.

CHEMICAL

THE ALCOHOL OF THE LUNG AIR AS AN INDEX OF ALCOHOL IN THE BLOOD*

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THE concentration of alcohol in the blood is now frequently determined from the concentration of alcohol in the air of the lungs. The values so obtained are used, not only as evidence of intoxication for the police court, but also as the basis for studies in regard to the drinking habits of motorists.

It is certain that the concentration of alcohol in the lung air varies directly with that in the blood. But there are differences of opinion as to the correct figure for the coefficient of distribution; that is, the figure by which the amount of alcohol in 1 c.c. of air should be multiplied to give the amount in 1 c.c. of blood. There is also a difference of opinion as to the procedure for determining the concentration of alcohol in the lung air. Uniform results are not obtained with the various methods now in use. This paper presents a critical study on both points.

The results obtained indicate that the coefficient now commonly employed is incorrect,¹ that the method now chiefly used for collecting lung air¹ does not give a correct value for the alveolar concentration of alcohol, and that consequently the results obtained for concentration in the blood and employed for juristic and statistical purposes may be correspondingly erroneous.

In 1934 two of us (H. W. H. and L. A. G.²) determined the coefficient of distribution of alcohol between air and water as 1:1,456 at 37.5° C., which we took as the temperature at which the distribution occurred in the lungs; we determined the coefficient for air and blood at the same temperature as 1:1,150. Harger, Lamb, and Hulpieu,¹ for an apparatus called the "drunkometer," chose a value of 2,000 for the coefficient of distribution of alcohol between air and blood. Haggard, Greenberg, and Cohen³ in turn criticized the accuracy of this procedure, both because of the high coefficient and because expired air was collected in a rubber bag. They were certain, from their own experience, that such collection led to the loss of considerable quantities of alcohol, and were of the opinion, but incorrectly as is here shown, that the loss was primarily by diffusion. In studies in which alveolar air was used in determining the concentration of alcohol in the blood, they were forced to employ trained persons who exhaled true alveolar air directly into the train of the analyzer.³ At that time this was the only method by which they were able to

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obtain, with the low coefficient which they employed, correct values for the concentration of alcohol in the blood as shown by direct determination from the blood.

In the course of the present study we have uncovered a feature which has not been previously taken into consideration in determining the concentration of alcohol in the blood from the concentration in the lung air, and which affords, in part at least, an explanation for the discrepancies between the coefficient used by two of us (H. W. H. and L. A. G.) and the one used by Harger, Lamb, and Hulpieu.¹ It is the retention of alcohol in the film of moisture which condenses on the surfaces of tubes and containers used in collecting expired air. When expired air at body temperature is cooled to room temperature in any container, water vapor condenses immediately as a mist, the particles of which rapidly coalesce to form a film on the inner surface of the container. The particles of the mist provide a large surface which permits a rapid equilibrium between the water and the alcohol vapor in the air. This equilibrium is established at a lower temperature, and, therefore, at a higher coefficient of distribution, than at body temperature. Although the total amount of water separated from the air is small, the high solubility of alcohol in water causes a marked reduction in the amount of alcohol in the air from which it condenses. The concentration in the air, as determined by analysis, is correspondingly lower than that which was actually present in the air before it left the lungs.

Since this feature, which must be considered in handling alcohol vapor, was unrecognized by two of us (H. W. H. and L. A. G.²) at the time we determined the coefficient distribution of alcohol between air and water and air and blood,³ these values have here been redetermined over a range of temperature from 20° to 40° C.

1. COEFFICIENT OF DISTRIBUTION OF ALCOHOL BETWEEN AIR AND WATER

The apparatus used in determining the coefficient of distribution of alcohol between air and water is shown in Fig. 1A. A stream of air flowing at the rate of 500 c.c. per minute, as determined by the calibrated manometer *M*, is washed through a saturated dichromate solution in concentrated sulfuric acid and passed successively through flasks *A*, *B*, *C*, and *D*, each containing 2 liters of alcohol solution of approximately 1 Gm. per liter. Dispersion of the air at the outlets in the flasks is obtained with fine earbonndum bubblers. The flasks, with all connections and stopcocks, are completely immersed in a water bath held at the desired temperature. The syringes, *E* and *F*, mounted on flasks *C* and *D*, permit withdrawal of water to determine the alcohol content by analysis. Flask *D* is open to the room through tube *G* which has a large bore; the pressure of air in flask *D* is not measurably above that of the room during the flow of air. The pressure in flask *C* can similarly be reduced by opening stopcock *I* when samples of air or water are withdrawn for analysis.

In determining the content of alcohol in the air of flask *D*, a measured volume of air at the rate of approximately 100 c.c. per minute is drawn through stopcocks *H* and *O* through an iodine pentoxide analyzer train.⁴ The volume of air passed through the analyzer is measured at atmospheric pressure and room temperature, but is corrected to the temperature in flask *D*.

An important feature of the apparatus shown in Fig. 1 is the provision made for evaporating condensed water vapor from the capillary tubes leading to the analyzer train. The condensate which forms, when the temperature of the bath is higher than that of the room, may hold considerable quantities of alcohol. After the desired amount of air for analysis is drawn through stopcock *II*, this stopcock is closed and stopcock *O* is turned so that a stream of fresh air will pass through the tube to the analyzer train. Any water which has condensed is evaporated and, together with the alcohol which it holds, is carried into the analyzer.

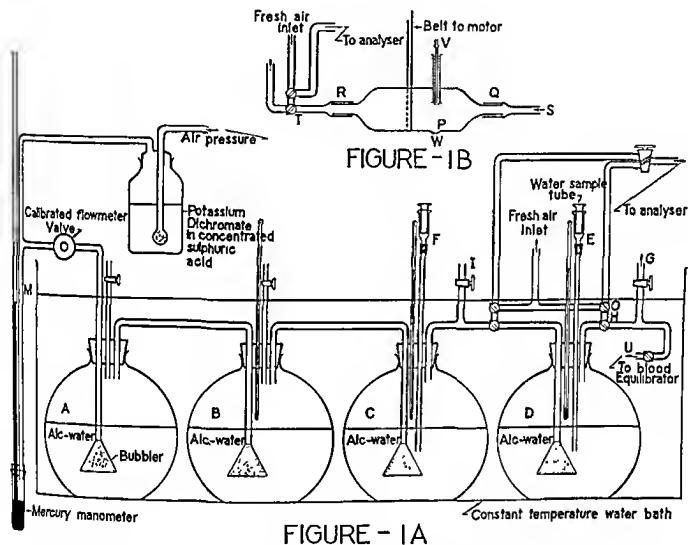


Fig. 1.—Apparatus for determining distribution of alcohol between air and fluids.

Completeness of equilibrium of alcohol between air and water in flask *D* was taken to exist when the concentration of alcohol in the air of flask *C*, as shown by analysis, corresponded exactly with that from flask *D*. In determining the coefficient of distribution at any temperature, the concentration of alcohol in the air of flask *D* is determined, and simultaneously water is withdrawn with syringe *E* and measured amounts are analyzed for alcohol. The calculation of the ratio is made as

$$(1) \quad 1: \frac{A' V}{A V'},$$

in which *A* is the amount of alcohol in milligrams in the air drawn through the analyzer train; *V*, the volume of air in cubic centimeters corrected to the temperature in flask *D*; and *A'*, the amount of alcohol in milligrams in the volume

of water, V' , in cubic centimeters, taken for analysis. Fig. 2 gives the values obtained for temperatures from 16.5° to 40° C.

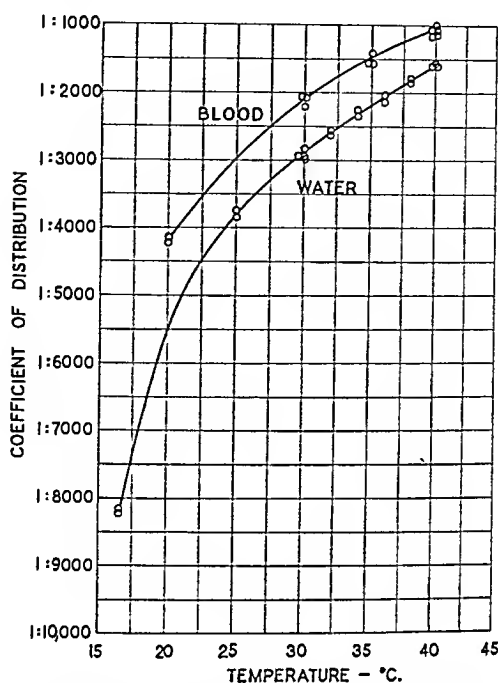


Fig. 2.—The distribution of alcohol between air and water, and between air and blood. Concentration of alcohol 0.06 to 0.30 per cent.

It should be possible to calculate the coefficient of distribution of alcohol between air and water from the values given in the literature for the vapor pressure of alcohol over mixtures of alcohol and water. These determinations have, however, been made with mixtures containing 10 per cent or more of alcohol by weight. The concentration found in the blood rarely exceeds 0.5 per cent. At temperatures as high as 40° C. the coefficient of distribution is considerably affected by such changes in concentration as that between 0.5 and 10 or 20 per cent. The coefficient of distribution is calculated from the vapor pressure from the ratio:

$$(2) \quad 1: \frac{10 Pd \times 76 \times 22.4 (273 + t)}{46 pp \times 273},$$

in which P is the per cent by weight of alcohol in the mixture; d the density of the mixture at temperature t ; and pp the partial pressure of the alcohol vapor over the mixture at this temperature. At 39.76° C., Wreusky⁵ gives the vapor pressure of alcohol over water containing 42 per cent by weight of alcohol as 63.0 mm.; 22 per cent as 44.55 mm.; 18.25 per cent as 40.1 mm.; and 15.92 per cent as 37.1 mm. The coefficients of distribution as calculated from these values are 1:2,590, 1:2,000, 1:1,865, and 1:1,760, respectively. From three determinations made at 40° C. on water containing 0.01 per cent of alcohol, we find coefficients (Fig. 2) of 1,580, 1,631, and 1,638.

2. COEFFICIENT OF DISTRIBUTION OF ALCOHOL BETWEEN AIR AND BLOOD

In determining the distribution of alcohol between air and blood, the apparatus shown in Fig. 1B is used in addition to that shown in Fig. 1A. The entire apparatus is immersed in the water bath of apparatus in Fig. 1A. Bulb *P* is of 100 c.c. capacity and is fitted by long ground joints, *Q* and *R*, to tubes *S* and *T*. Tube *S* is connected under water to tube *U* of apparatus 1A, and a stream of air containing alcohol vapor from the flasks *A* to *D* is passed through bulb *B* at the rate of 750 c.c. per minute and discharged through tube *T*. Two cubic centimeters of blood are delivered into the bulb through tube *V* which projects to the middle of the bulb and is closed by a glass-tipped stopper. The bulb is rotated at the rate of 10 to 15 r.p.m.; the blood is thus spread in a thin film on the wall of the bulb for equilibration with the air containing alcohol.

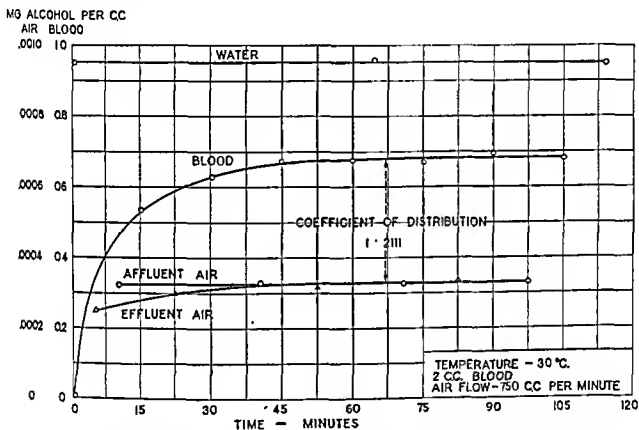


Fig. 3.—The concentrations of alcohol in air and blood during equilibration in apparatus shown in Fig. 1A and B.

There is no evaporation of the blood since the air-leaving flask *D* is fully saturated with water at the temperature of the bath. In withdrawing blood for analysis, the rotation of the bulb is stopped with tube *V* in a vertical position, as shown, and projecting above the level of the water in the bath. Blood collects in the depression *W* and is withdrawn with a pipette inserted through tube *V*. Affluent air of the bulb is obtained for analysis through stopcock *H* of apparatus in Fig. 1A and effluent air through stopcock *T*. Precautions are taken, as described, to remove condensed water vapor.

In determining the distribution of alcohol between air and blood, the flow of air was continued for ninety to one hundred and twenty minutes. In order to be certain that equilibrium was complete, analyses were made at intervals of the alcohol in the water of flask *D*, in the affluent and effluent airs of bulb *P*, and in the blood. The values obtained in one such determination are shown in Fig. 3. Equilibration was complete here in sixty minutes. As a further

check upon the accuracy of the method used, a series of determinations was made with water instead of blood; the values obtained corresponded exactly with those determined as described in the previous section and given as the curve for water in Fig. 2.

Fig. 2 gives the values obtained for the coefficient of distribution of alcohol between air and blood at temperatures of 20° to 40° C. The values at 35° and 40° C. are 1:1,550 and 1:1,124, respectively. The values previously determined by two of us (H. W. H. and L. A. G.²) for these temperatures are 1:1,204 and 1:1,130. The value of 1:2,000, employed by Harger, Lamb, and Hulpieu¹ corresponds, from our determinations here, to a temperature of 31° C.

This value of 1:2,000 was not determined in vitro by Harger, Lamb, and Hulpieu, but is that given by Liljestrand and Linde.⁶ These latter investigators obtained the value empirically from determinations made on expired air and blood. No consideration was given to the loss of alcohol in condensed water vapor in collecting the air. Moreover, they are of the opinion that mixed expired air and alveolar air contain the same amounts of alcohol, a feature which Harger, Lamb, and Hulpieu were unwilling to accept, although they employed their coefficient of distribution.

3. CONCENTRATION OF ALCOHOL IN THE ALVEOLAR AND VENOUS AIRS

It is difficult to obtain on untrained subjects, particularly if they are partially intoxicated, a reliable sample of alveolar air. For these reasons, Harger, Lamb, and Hulpieu¹ collected mixed expired air, determined both the concentration of alcohol and the per cent of carbon dioxide, and converted the former to what they assumed to be the alveolar concentration of alcohol on the basis of a constant 5.5 per cent of carbon dioxide in alveolar air. This procedure involves the assumption that carbon dioxide and alcohol diffuse with equal rapidity into the air of the respiratory dead space, an assumption which we shall later demonstrate to be incorrect. In obtaining the alveolar concentration of alcohol we³ have previously employed trained subjects and taken, as alveolar air, the last third of expiration as in the method of Haldane and Priestley.⁷ We find now that this procedure is unnecessary and that the venous air⁸ obtained by a short period of rebreathing gives, for both trained and untrained subjects, a concentration of alcohol that is nearly identical with that obtained from alveolar air. These same relations have been shown previously to hold for carbon monoxide present in the blood.⁹

A consideration of the principles of elimination of alcohol shows the theoretical validity of the procedure. Because of its high solubility, alcohol is eliminated only in small amounts in the expired air; the concentrations in the pulmonary venous and arterial bloods, unlike the concentrations of carbon dioxide and oxygen, are only slightly different. The alcohol in the blood reaching the lungs is distributed between the respective volumes of air and blood in the relation of the distribution coefficient.^{10, 11} Therefore,

$$(3) \quad Ac = \frac{B + \frac{L}{C}}{V_c B},$$

in which A_c and V_c are the concentrations of alcohol in grams per liter in the arterial blood leaving the lungs and the venous blood reaching the lungs; B , the volume of blood circulated through the lungs in time t ; L , the volume of pulmonary ventilation in this time; and C , the coefficient of distribution of alcohol between blood and air with the concentration in air taken as 1.

During rest the volume of the circulation through the lungs and the volume of the pulmonary ventilation are approximately the same. On this basis, the relation between the concentrations of alcohol in the arterial and venous bloods at a distribution coefficient of 1:1,300 would be 1:1 00077 (equation 3). The error introduced in the determination of the arterial concentration would, therefore, be 0.077 per cent. Even wide variations in blood flow and volume of pulmonary ventilation would not introduce an appreciable error; thus if the ventilation were ten times as great as the blood flow, the theoretical error as calculated would be increased from 0.077 to only 0.77 per cent.

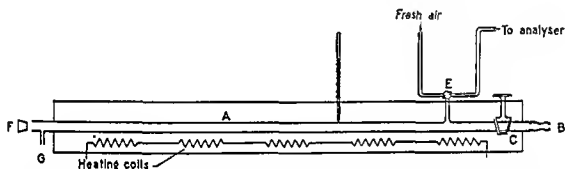


Fig. 4.—Apparatus for determining the concentration of alcohol in the alveolar and venous air

The apparatus shown in Fig. 4 is used to determine the concentration of alcohol in both alveolar and venous air. It consists of a glass tube, A , of 2.5 cm. bore, 2 meters long, contained in an asbestos lined wooden box, 15 cm. in sectional diameter. The tube projects a short distance from the ends of the box and is fitted at one end with a glass mouthpiece, B , made tight with a ground joint and removable for sterilization with dry heat. The stopcock C , of 1.25 cm. bore, is controlled from outside the box and serves to close off the tube after the sample of lung air has been collected. To prevent condensation of water vapor with loss of alcohol, as discussed in the next section of this paper, the tube is kept at a temperature slightly above 40° C. by heating coils; the current is regulated by a rheostat.

Alveolar air can be collected in the tube as in the procedure described by Haldane and Priestley.⁷ Following a normal inspiration, a deep expiration is made and stopcock C is closed. Venous air is collected by having the subject wear a nose clip and rebreathe from the tube six times; stopcock C is closed at the end of the last expiration. To determine the concentration of alcohol in the air collected, stopcock E is opened and 100 to 200 c.c. of air are drawn from the tube into the analyzer train by water displaced as described for the apparatus shown in Fig. 1A; suitable correction is made for the difference in temperature of the air in the tube and that in the room. Stopcock E is then turned to permit a flow of fresh air, obtained outside the room, to flush the tubes leading to the analyzer train and to remove any condensed water vapor. The expired air is flushed from tube A by opening stopcock C , inserting stopper F , and drawing

a brisk current of air through tube *G* which is connected to a suction pump. The apparatus described here can be assembled in more compact form by having tube *A* bent into a coil and enclosed in a square box.

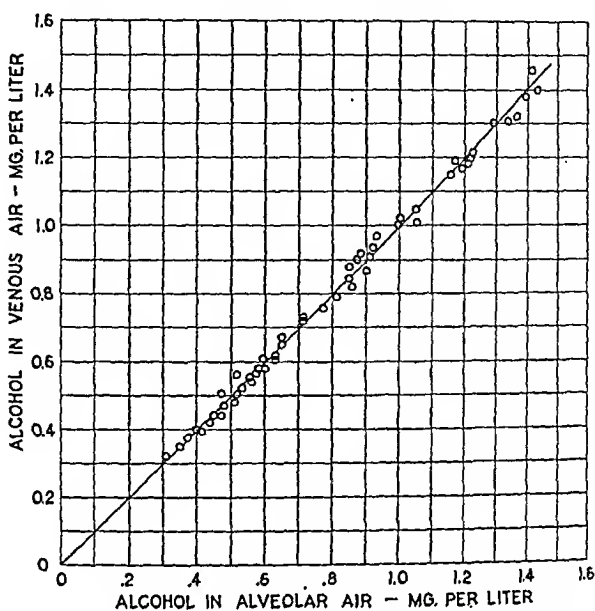


Fig. 5.—Correlation between the concentration of alcohol in alveolar and venous airs.

Fifty-three pairs of comparative determinations were made of the concentrations of alcohol in the alveolar and venous airs of 5 trained subjects. They each drank from 30 to 150 c.c. of alcohol diluted to 50 per cent by volume with water. At intervals thereafter the concentration of alcohol in the alveolar air was determined and immediately following, that in the venous air. Fig. 5 presents the values obtained; each dot is made at the point of intersection of lines drawn from the ordinate value for the concentration in the venous air and from the abscissa value for that in the alveolar air of corresponding determinations. The average of all the values obtained for the alveolar air was 0.814, and for the venous air, 0.823 mg. per liter. The maximum variation in any single pair of determinations was less than 5 per cent.

4. CONCENTRATION OF ALCOHOL IN THE MIXED EXPIRED AIR

It is possible, as is demonstrated in the next section of this paper, to collect expired air in a rubber bag without appreciable loss of alcohol during the first five minutes, provided the bag is kept warmed to a temperature of 40° C. to prevent condensation. In the study here a rubber bag was placed in an incubator kept slightly above this temperature; the short glass tube leading to the bag, and used as a mouthpiece by the subject, was passed through the side of the incubator and was warmed with a small heating coil. In obtaining mixed expired air the subject, previously given alcohol, exhaled into the bag; the bag was emptied and a second expiration was collected. The mouthpiece was then closed and a measured sample of the air was drawn into the analyzer train with precautions, as previously described, to remove the condensed water vapor.

Table I gives a series of values obtained from one subject for the concentration of alcohol and per cent of carbon dioxide in the mixed expired and alveolar airs. Nine similar series made with this subject and with four other subjects gave the same results within the ordinary limits of individual variation.

TABLE I

SUBJECT GIVEN BY STOMACH 110 C.C. OF ETHYL ALCOHOL DILUTED TO 50 PER CENT WITH WATER

TIME AFTER GIVING ALCOHOL (HR.)	ALCOHOL		CARBON DIOXIDE		
	MIXED EXPIRED AIR (MG./L.)	ALVEOLAR AIR (MG./L.)	EXPIRED AIR (%)	ALVEOLAR AIR (%)	ALCOHOL IN MIXED EXPIRED AIR $\times \frac{\text{CO}_2 \text{ ALVEOLAR}}{\text{CO}_2 \text{ EXPIRED}}$ (MG./L.)
1	1.01	1.15	4.1	5.6	1.38
2	0.97	1.15	3.8	5.5	1.40
3	0.85	1.06	3.9	5.8	1.26
4	0.71	0.90	4.0	5.6	0.99
5	0.63	0.77	3.9	5.8	0.93
6	0.48	0.61	3.7	5.4	0.70
7	0.40	0.45	3.9	5.6	0.58

The last column of Table I gives the concentration of alcohol calculated for alveolar air as in the procedure followed by Harger, Lamb, and Hulpieu,¹ i.e., concentration of alcohol in mixed expired air $\times \frac{\text{CO}_2 \text{ in alveolar air}}{\text{CO}_2 \text{ in mixed expired air}}$. In each instance this calculated value is higher than that obtained by direct measurement: the average difference is 18 per cent, and the minimum and maximum differences are 9 and 23 per cent. These findings suggest that alcohol diffuses more rapidly from the surface of the respiratory passages into the air in the respiratory dead space than does carbon dioxide. The diffusion of alcohol from these surfaces does not affect the concentration of alcohol obtained directly from the alveolar or venous air, but only from the mixed expired air, approximately one-third the volume of which comes from the virtual dead space. Haldane¹² has demonstrated that the carbon dioxide which diffuses from the respiratory passages enters the mixed expired air, but not the exhaled alveolar air. In rebreathing, as in the procedure used here to obtain venous air, equilibrium of alcohol is reached throughout the entire respiratory tract. We have demonstrated that the concentrations of alcohol in the alveolar and venous airs are nearly identical (Fig. 5).

The extensive diffusion of alcohol into the air in the respiratory dead space has been previously observed by Liljestrand and Linde;⁶ they found that mixed expired air contains nearly as much alcohol as the alveolar air. As seen from the values given in Table I, the concentration of alcohol in the alveolar air is, however, higher than in the mixed expired air. The average difference is 21 per cent.

A series of experiments was carried out here to demonstrate that the diffusion of alcohol into the air of the respiratory dead space is more rapid than that of carbon dioxide. For this purpose the mouth air was used, as in the studies of Henderson and Stehle¹³ on the diffusion of carbon dioxide and oxygen. A subject, previously given alcohol, inhaled and then immediately closed off the

mouth at the pharynx and breathed through the nose. A glass tube was held between the lips, and the mouth was inflated with 75 c.c. of air by means of a hand bulb attached to the tube; the mouth was then emptied and reinflated. The air was held for two minutes, and the concentration of alcohol and the per cent of carbon dioxide in it were then determined. Immediately thereafter the concentration of alcohol and per cent of carbon dioxide in the alveolar air were determined. In a series of four such experiments the concentrations of alcohol in the mouth air were 42.4, 43.2, 41.7, and 44.9 per cent of that in the alveolar air; the per cents of carbon dioxide in the mouth air were only 16.3, 14.6, 15.1, and 17.2 of that in the alveolar air. In a second series of experiments, in which the air was held five minutes instead of two, the differences in rate of diffusion were still evident, although less marked; the average value for the concentration of alcohol was 46.2 per cent, and the carbon dioxide was 36.1 per cent of that in the alveolar air.

The concentration of alcohol in the mixed expired air is less than that in the alveolar air by approximately the same amount that it is exceeded by the concentration calculated for the alveolar on the basis of the per cents of carbon dioxide (columns 2, 3, and 6, Table I). It would appear then to make little difference, so far as accuracy is concerned, which of the two values is taken in calculating the concentration of alcohol in the blood; the error would be the same, but in opposite directions. An error of 20 per cent in calculating the concentration of alcohol in the blood from that in the lung air is probably not serious if such determinations are intended only as rough clinical approximations or are to be used, as they often have been used, in police tests to coerce the suspect into a confession of having drunk immoderate amounts of alcohol.^{1, 11}

This, however, is not the only error resulting from the procedure as now generally used. A further and widely variable error is introduced by this method of collecting the expired air and will be discussed in the following section.

5. LOSS OF ALCOHOL IN CONDENSED WATER VAPOR

When expired air is collected in containers or passed through tubes at a temperature lower than that of the body, alcohol, as we have stated, is removed in the condensed water vapor. This loss, and the corresponding error introduced into determinations of the concentration of alcohol in respired air, is not constant, but varies with the temperature of the container. The concentration of carbon dioxide in the air, because of the low solubility of this gas, is not appreciably affected by the condensation of water vapor.

The following experiments demonstrate the loss of alcohol in condensed water vapor. Air at 40° C. containing alcohol and saturated with water vapor was run from outlet *G* of flask *D*, of the apparatus shown in Fig. 1A, into a rubber bag. The bag was placed in an incubator so that it could be kept at any temperature desired. The short glass connection between the source of alcohol vapor and the bag was warmed with an electric heating coil. After 1 liter of air had been collected, the bag was emptied and again filled with this quantity of air. Samples of air were drawn from the bag at two, five, fifteen, and thirty minutes, and the concentration of alcohol was determined. The values obtained are shown in Fig. 6.

When the bag in which the air was collected was warmed to 40°C . (curve A, Fig. 6), the loss of alcohol was slow and nearly uniform; in five minutes the concentration had fallen 3 per cent, and in thirty minutes, 13 per cent. When the bag was kept at a temperature of 20°C . (curve C, Fig. 6), there was an immediate sharp drop in concentration, followed by a slow progressive drop; in two minutes the concentration had fallen 18 per cent; in five minutes, 26 per cent; in fifteen minutes, 30 per cent; and in thirty minutes, 34 per cent. When the bag was cooled to 10°C . (curve D, Fig. 6), the decreases in concentration at

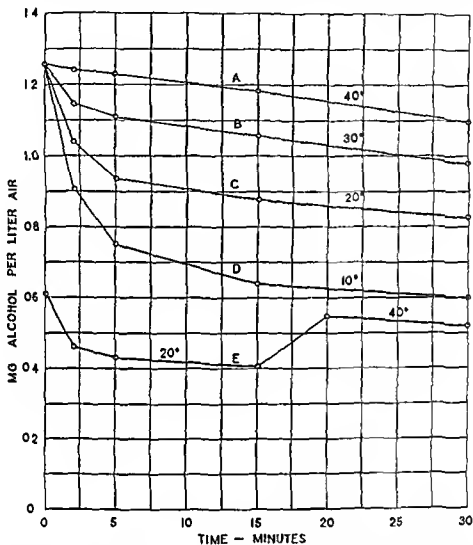


Fig. 6.—Loss of alcohol from lung air in condensed water vapor at different temperatures.

the corresponding times were 28, 41, 50, and 52 per cent. In a final series of determinations (curve E, Fig. 6), the bag was first kept at 20°C . for fifteen minutes; during this time the concentration fell 32 per cent. The bag was then warmed to 40°C . to evaporate the condensed water vapor; the concentration of alcohol rose, reaching a value only 14 per cent below the initial concentration.

From the results obtained here it is evident that if expired air is collected, as is now the practice,¹ errors as great as, or even greater than, 50 per cent are to be expected. Such a collection is made from motorists at the roadside, under a wide range of daily and seasonal temperatures, and no precautions are taken to prevent condensation of water vapor from the breath. The error from condensation can be avoided only by having the receptacle in which the air is collected at a temperature sufficiently high to prevent condensation of moisture, as is done in the apparatus shown in Fig. 4.

Loss of alcohol in condensed water vapor occurs, not only when rubber bags are used to collect expired air, but also when the air is passed through glass or

metal tubes. This fact is demonstrated here by a series of determinations of the concentration of alcohol in alveolar air (Haldane and Priestley) made with the apparatus shown in Fig. 4. In separate determinations the tube was kept at 10°, 20°, 40°, and 50° C. One trained subject was used and ten determinations were made at each temperature. The coefficient of distribution of alcohol between air and blood was calculated from the concentration of alcohol obtained in the alveolar air and that in blood drawn simultaneously. At 10° C. the average value found for the coefficient was 3,123; at 20° C., 1,819; and at 40° C., 1,304. On increasing the temperature of the tube to 50° C., no further change occurred in the coefficient, which at this temperature was 1,306.

6. COEFFICIENT OF DISTRIBUTION AS DETERMINED ON 100 SUBJECTS

One hundred different subjects were either given alcohol or were brought to the laboratory after they had taken alcohol. No comparison between the concentration of alcohol in the blood and venous air was made until it was certain, from repeated determinations on the blood, that the concentration of alcohol had reached its maximum and had decreased over a period of at least one hour. This feature is important in using the concentration of alcohol in the capillary or venous blood as an index of that in the arterial blood. When absorption and distribution of alcohol are complete, the concentration of alcohol in blood from all sources corresponds closely, but prior to this time, exact agreement does not exist and appreciable errors may be introduced into the calculation of the distribution coefficient.^{2, 15}

The blood of the subjects was tested for acetone by the method described by Shipley and Long;¹⁶ no appreciable amount was found in any instance. This procedure was followed, since iodine pentoxide, used for the analysis of alcohol, reacts to acetone. In the analysis of blood the acetone can be removed with Mariott Scott-Wilson reagent,¹¹ but similar removal cannot be made from the respired air. In determinations made on more than 200 subjects given alcohol, we have found no amount of acetone in the blood sufficient to introduce any error into the determination of alcohol. An equal number of determinations made on the expired air of these subjects before they were given alcohol showed a similar absence of oxidizable material when the sample taken for analysis was not larger than 200 c.c. This result was obtained even on subjects who had eaten onions or candy containing essential oils. From them, and also from subjects with foul breath, a faint blue coloration is obtained in the starch iodide solution used for collecting the iodine liberated from the pentoxide when, and only when, more than 1 liter of expired air is passed through the analyzer train;¹⁷ this amount is five to ten times that used in the analyses reported here.

The concentrations of alcohol in the bloods of the one hundred subjects used in determining the coefficient of distribution ranged between 0.22 and 2.88 mg. per cubic centimeter. The concentration of alcohol in the venous air was determined simultaneously with that in the blood. The values calculated for the coefficients of distribution of alcohol between air and blood are shown as dots in Fig. 7. The average for the series was 1:1,307, with extremes of 1:1,180 to 1:1,307, variations of -9.7 and +8.9 per cent.

Harger, Lamb, and Hulpieu¹ have reported a similar series of determinations in which the "drunkometer" was used to obtain the concentration of alcohol in the expired air from which that in the alveolar was calculated on the basis of the per cents of carbon dioxide in expired and alveolar air. The values they obtained are shown in Fig. 7 as circles; the average value is approximately 2,000, but the extremes are 1:1,220 to 1:4,200, variations of -39 and +110 per cent. Such variations would appear extreme even for medicolegal approximations.

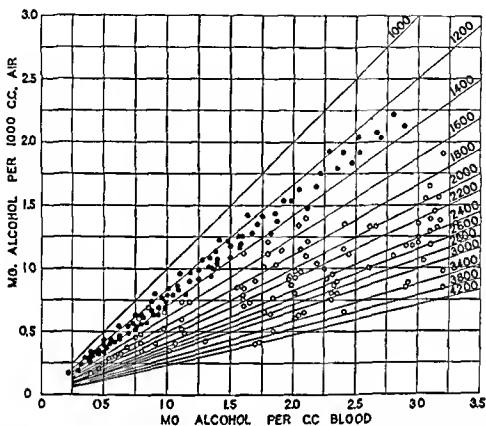


Fig. 7.—The coefficient of distribution of alcohol between blood and lung air. Dots, values as determined here; circles, values as reported by Harger, Lamb, and Hulpieu.

7. TEMPERATURE AT WHICH THE DISTRIBUTION OF ALCOHOL OCCURS IN THE LUNGS

The coefficient of distribution of 1:1,307, as given in the previous section, corresponds to a temperature for the distribution of 38° C. (see Fig. 2). This temperature is very nearly that of the blood flowing through the lungs.^{18, 19} No measurements have ever been made of the temperature of the air in the lungs, and few reliable ones have been made of the temperature of the air leaving the lungs when not mixed with air in the respiratory dead space. The most recent and precise are those made by Seeley.²⁰ He finds that when the air breathed is at 20° C., the temperature of the exhaled air measured at the larynx is 35° C.; when the air breathed is 10° C., the temperature of the exhaled air falls to 34° C., and when breathed at 40° C. it rises to 36° C. It is certain that the temperature of the expired air does not precisely represent that in the lungs. If it did, the air in the alveolar spaces would be indicated as having a lower temperature than the blood passing through the lungs.

The air in the respiratory dead space, which is motionless between the times of inhalation and exhalation, comes into partial equilibrium, as we have shown, with the alcohol on the surface of the respiratory passages; this alcohol appears

in the air of the first part of expiration. It is improbable, for reasons which we have previously indicated, that during expiration the rapidly moving alveolar air either receives or gives up alcohol to surface tissues which may be at a lower temperature than the lungs. We have, however, investigated this point. On rebreathing from the tube of the apparatus shown in Fig. 4, to obtain venous air, the inhaled air becomes warmed to nearly 40° C. We have already shown (Fig. 5) that the concentration of alcohol in the alveolar air, after inhaling air at ordinary room temperature, is nearly identical with that of the venous air. This fact suggests that the temperature of the expired air has no influence upon the concentration of alcohol in the alveolar air. These observations were extended to include room temperatures of 10° and 40° C. Neither temperature affected the concentration of alcohol, as determined in the alveolar air.

When the alveolar air was exhaled into the tube (apparatus Fig. 4), heated to 40° C. and allowed to remain there, no fog and no condensation occurred; when the temperature of the tube was lowered to 35° C., there was a slight, but definite, fog; and when the experiment was repeated at a temperature of 30° C., there was a heavy fog, with condensation of a film of water upon the interior of the tube. This observation indicates that the alveolar air is saturated with water vapor at a temperature above 35° C. If, then, during expiration, the air from the depths of the lungs does not lose water in the respiratory passages, even at their lower temperatures, it is highly probable that it would not lose alcohol.

More precise measurement of the water vapor was obtained by direct determination from the alveolar and venous airs. A U-tube containing 70 Gm. of anhydrous magnesium perchlorate was attached to the tube from stopcock E of the apparatus shown in Fig. 4. The glass connections were warmed with a heating coil to prevent condensation. Two hundred cubic centimeters of alveolar or venous air were drawn through the U-tube, and the gain in weight was determined. The values obtained for five determinations on one subject for alveolar air with inspired air at 20° C. were: 43.0, 43.1, 44.0, 42.6, and 43.2 mg. per liter, the average of which corresponds to the amount of water in air fully saturated at a temperature of 37.2° C. The values obtained from the venous air with the rebreathed air at 40° C. were: 41.7, 44.2, 43.2, 44.0, and 43.4 mg. per liter of air.

CONCLUSIONS

1. The coefficient of distribution of alcohol between air and blood, now widely employed, is that assigned by Harger, Lamb, and Hulpieu, viz., 1:2,000. We find that the correct value, as determined both *in vitro* and *in vivo*, is 1:1,300.
2. The discrepancy is, in part, due to the loss of alcohol in condensed water vapor in containers used to collect expired air. This loss has been demonstrated.
3. The concentration of alcohol in lung air cannot be correctly calculated from the concentration in mixed expired air on the basis of carbon dioxide content of these airs. Alcohol diffuses more rapidly into the air of the respiratory dead space than does carbon dioxide. The diffusion does not affect the concentration in the alveolar or venous air.
4. The concentration of alcohol in the venous air corresponds closely to that in the alveolar air. A method is described for obtaining venous and alveolar air without loss of alcohol.

5. The temperature at which the distribution of alcohol between air and blood takes place in the body is that of the blood in the lungs

6. The procedure now widely used in medicolegal investigation and statistical studies for determining the concentration of alcohol in the blood from that in the expired air is subject to serious errors. It is, however, possible to determine accurately the concentration in the blood from that in the lung air.

REFERENCES

1. Harger, R. N., Lamb, E. B., and Hulpieu, H. R.: A Rapid Chemical Test for Intoxication Employing Breath. A New Reagent for Alcohol and a Procedure for Estimating the Concentration of Alcohol in the Body From the Ratio of Alcohol to Carbon Dioxide in the Breath. *J. A. M. A.* 110: 779, 1938.
2. Haggard, H. W., and Greenberg, L. A.: Studies in the Absorption, Distribution and Elimination of Ethyl Alcohol. II. The Excretion of Alcohol in Urine and Expired Air; and the Distribution of Alcohol Between Air and Water, Blood and Urine, *J. Pharmacol. & Exper. Therap.* 52: 150, 1934.
3. Haggard, H. W., Greenberg, L. A., and Cohen, L. H.: Quantitative Differences in the Effects of Aleoholic Beverages, *New England J. Med.* 219: 466, 1938.
4. Haggard, H. W., and Greenberg, L. A.: Studies in the Absorption, Distribution and Elimination of Ethyl Alcohol. I. The Quantitative Determination of Ethyl Alcohol in Air, Blood, and Urine by Means of Iodine Pentoxide, *J. Pharmacol. & Exper. Therap.* 52: 137, 1934.
5. Wrewsky, M.: Über Zusammensetzung und Spannung des Dampfes binärer Flüssigkeitsgemische. Erster Teil, *Ztschr. f. physiol. Chem.* 81: 1, 1912.
6. Liljestrang, G., and Linde, P.: Über die Ausscheidung des Alkohols mit der Expirationsluft, *Skandinav. Arch. f. Physiol.* 60: 273, 1930.
7. Haldane, J. S., and Priestley, J. G.: The Regulation of the Lung Ventilation, *J. Physiol.* 32: 225, 1905.
8. Henderson, Y., and Prince, A. L.: Applications of Gas Analysis. II. The CO₂ Tension of the Venous Blood and the Circulation Rate, *J. Biol. Chem.* 32: 325, 1917.
9. Henderson, Y., Haggard, H. W., Teague, M. C., Prince, A. L., and Wunderlich, R. M.: Physiological Effects of Automobile Exhaust Gas and Standards of Ventilation for Brief Exposures, *J. Indust. Hyg.* 3: 79, 1921.
10. Haggard, H. W.: The Absorption, Distribution and Elimination of Ethyl Ether. I. The Amount of Ether Absorbed in Relation to the Concentration Inhaled and Its Fate in the Body, *J. Biol. Chem.* 59: 737, 1924.
11. Haggard, H. W., and Greenberg, L. A.: Studies in the Absorption, Distribution and Elimination of Alcohol. IV. The Elimination of Methyl Alcohol, *J. Pharmacol. & Exper. Therap.* 66: 479, 1939.
12. Haldane, J. S.: *Respiration*, New Haven, 1922, Yale Univ. Press, p 38.
13. Henderson, Y., and Stehle, R. L.: Gas Tensions in the Tissues of the Mouth, *J. Biol. Chem.* 38: 67, 1919.
14. Committee Report, *J. A. M. A.* 112: 2164, 1939.
15. Haggard, H. W., Greenberg, L. A., and Rakieten, N.: Studies on the Absorption, Distribution and Elimination of Alcohol. VI. The Principles Governing the Concentration of Alcohol in the Blood and the Concentration Causing Respiratory Failure, *J. Pharmacol. & Exper. Therap.* 69: 252, 1940.
16. Shipley, R. A., and Long, C. N. H.: Studies on the Ketogenic Activity of the Anterior Pituitary. I. The Relation of Ketonaemia to Ketonuria in the Rat. II. A Method for the Assay of the Ketogenic Activity. III. The Nature of the Ketogenic Principle, *Biochem. J.* 32: 2242, 1938.
17. Haggard, H. W., and Greenberg, L. A.: Breath Odors From Alliacous Substances, *J. A. M. A.* 104: 2160, 1935.
18. Wiggers, C. J., and Orian, O.: The Circulatory Changes During Hyperthermia Produced by Short Radio Waves (Radiothermia), *Am. J. Physiol.* 100: 614, 1932.
19. Nedzel, A. J.: Temperature Changes in Trachea and Kidney in Relation to Heat and Cold on Skin, *Proc. Soc. Exper. Biol. & Med.* 30: 691, 1933.
20. Seeley, L. E.: Study of Changes in the Temperature Water Vapor Content of Expired Air in the Nasal Cavity, *A. S. H. V. E.* 12: 377, 1940.

A CLINICAL METHOD FOR THE DETERMINATION OF ASCORBIC ACID IN BLOOD PLASMA AND URINE*

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MINDLIN and Butler¹ have developed a method for the determination of plasma ascorbic acid, measuring photoelectrically the excess dye remaining after the reaction between ascorbic acid in a metaphosphoric acid filtrate and 2,6 dichlorophenolindophenol. In avoiding a titrimetric procedure in strong acid solution which allows considerable error due both to slow reduction by other materials and to decomposition of the oxidized dye, the method represents a real advance. The method of Bessey² extends the usefulness of this procedure by eliminating the necessity of absolutely clear and colorless filtrates. The preparation of such filtrates in the first method requires considerable care and time in filtering; in the second method two readings of the photoelectric device are necessary for each sample. In both procedures the photoelectric readings must be completed immediately after each addition of dye, with due care exercised in observing possible "drifts" of the reading due to the presence of slowly reducing materials, all of which is very time-consuming in serial analysis. The finding of Bukatsch³ that oxidized 2,6 dichlorophenolindophenol can be quantitatively extracted from acid solution with xylene has led to an accurate, rapid clinical method. The method developed includes all the advantages of the procedures described, does not require special care in the preparation of the filtrate, and requires only a single photoelectric reading for each sample. The dye is allowed contact with the ascorbic acid for the short time necessary for reaction, then extracted into xylene, where it is no longer subjected to an acid medium or to slowly reducing substances, and is, therefore, completely stable for hours. The extraction involves a simple shaking with xylene and brief centrifugation. Because of the stability feature, as many samples as desired can be treated with the dye and extracted, and when all are completed, the color of the xylene layers measured as a unit in the photoelectric device. The separation of the chemical work from the photoelectric measurements saves time in its greater convenience. The stability of the dye in xylene permits the reliable use of the visual colorimeter in the absence of photoelectric devices.

The method to be described was developed with the view of increasing the ease and speed of plasma ascorbic acid determinations in serial analysis required in a recent research project.

DETERMINATION

Apparatus and Reagents.—Any photoelectric colorimeter, or, as employed in this work, a Coleman "DM" photoelectric spectrophotometer.

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Metaphosphoric acid. Six grams of clear sticks of glacial metaphosphoric acid, dissolved in 100 c.c. of water and filtered. This reagent is serviceable for eight to ten days if kept in the icebox.

Dye solution. Approximately 12 mg. of 2,6 dichlorophenolindophenol dissolved in 200 c.c. of warm distilled water, cooled and filtered. This reagent is satisfactory for eight to ten days if kept in the icebox.

Sodium hydroxide. An approximately 0.8 N solution made by dissolving 32 Gm. of sodium hydroxide in 1 liter of water.

Phosphate-citrate buffer. Approximately pH 4.0. Mix 30 c.c. of 0.2 M disodium phosphate and 50 c.c. of 0.1 M citric acid.

Bromeresol green 0.04 per cent. Add 14.3 c.c. of 0.01 N sodium hydroxide to 0.1 Gm. of bromeresol green and make up to 250 c.c. with water.

Xylene, C.P.

Method.—Blood for analysis should be collected (approximately 7 c.c.) with a dry syringe and kept in thoroughly clean pyrex tubes containing oxalate (about 20 mg.) as an anticoagulant. Excessive shaking and other causes of hemolysis are to be avoided. The tubes are chilled after collection and analysis is started within five hours. The blood is centrifuged and the plasma is withdrawn. Three cubic centimeters of plasma are measured into a test tube, followed by 3 c.c. of distilled water and 6 c.c. of 6 per cent metaphosphoric acid. After thorough mixing and allowing to stand for fifteen minutes, the tubes are centrifuged at 2,500 r.p.m. for fifteen minutes. The metaphosphoric acid filtrate may be kept for several hours in the cold without loss of ascorbic acid. A pipette is inserted into the supernatant fluid and exactly 8.0 c.c. of filtrate are removed to a 30 c.c. test tube. Two drops of bromeresol green indicator are added and 0.8 N sodium hydroxide is added dropwise with shaking until a green color is reached, then approximately 1 c.c. of the phosphate citrate buffer is added. Exactly 2.0 c.c. of the dye solution are added, the tube is revolved for mixing, the solution is layered with 12.0 c.c. of xylene, corked, and shaken for ten to fifteen seconds. Only fifteen to thirty seconds need elapse between addition of the dye and extraction in order to avoid slow, interfering reactions. The amount of dye prescribed in this test is sufficient to react with approximately 0.05 mg. of ascorbic acid or a plasma ascorbic acid of 2.5 mg. per cent. If more than this is encountered and the dye is completely reduced, accuracy is not sacrificed by adding a further 1.0 or 2.0 c.c. of dye at this stage. The tube is centrifuged briefly to separate the layers. Occasionally, and more often on too vigorous a shaking, the two layers do not separate well. In this case, after the first centrifugation, the xylene layer should be stirred briefly with a glass rod and again centrifuged. At the end of a series of analyses a "control" tube should be prepared, containing 8 c.c. of water, 1 c.c. of buffer, and exactly 2.0 c.c. of dye, followed by xylene and extraction.

The xylene layer may now be poured off easily and at any time within several hours into the special cells of the photoelectric device. In the case of the spectrophotometer, with xylene in the "solvent" tube, the transmission of the "unknown" and "control" tubes are determined at wave length $\lambda = 500 \text{ m}\mu$. (In the Evelyn or other photoelectric colorimeters the filter trans-

mitting maximally at 500 $m\mu$ should be used.) The difference in the logarithms of these two transmissions is directly proportional to the amount of ascorbic acid originally present, that is,

$$C = k(\log I^0/I \text{ control} - \log I^0/I \text{ unknown}) = k(\Delta \log).$$

Entirely similar relations are given in Bessey's paper for the case of the Evelyn colorimeter. The value of K must be determined only once for the prescribed conditions and for the instrument used, with a carefully weighed or iodine standardized ascorbic acid solution. Thus in this case a $\log = 0.064$ was equivalent to 0.01 mg. of ascorbic acid, hence the milligrams of vitamin C involved in a given determination would be

$$C = \frac{\Delta \log}{0.064} \times 0.01.$$

Since the 8 c.c. of filtrate represents 2.0 c.c. of plasma, then in milligrams per cent of plasma ascorbic acid

$$C = \frac{\Delta \log}{0.064} \times 0.01 \times 50 = 7.81 (\Delta \log).$$

If the visual colorimeter is used, various dye controls must be prepared as above in order to permit accurate comparison with the widely different ascorbic acid values encountered in routine analysis. Also, each dye solution utilized must be standardized against a carefully prepared ascorbic acid solution. If reduction of the amount of dye prescribed is great, more favorable colorimetry is obtained by adding a greater excess of dye. Visual colorimetry cannot, however, provide the accuracy obtained by photoelectric measurement.

TABLE I
REPRODUCIBILITY OF METHOD AND COMPARISON WITH BESSEY'S² PROCEDURE OF
PLASMA ASCORBIC ACID DETERMINATION

SAMPLE	PRESENT METHOD (MG. %)	BESSEY'S METHOD (MG. %)	AVERAGE % DIFFERENCE
Blood 1	0.74	0.77	4.6
	0.70	0.72	
	0.71	0.76	
Blood 2	1.08	1.16	4.3
	1.06	1.12	
	1.11	1.11	
Blood 3	0.38	0.41	5.0
	0.42	0.44	
	0.39	0.40	
Blood 4*	1.79	1.83	2.8
	1.82	1.88	
	1.82	1.87	

*Taken after administration of vitamin C.

The same method may be readily applied to urine. Equal volumes of freshly voided urine and 6 per cent metaphosphoric acid are mixed and filtered. An appropriate volume of this filtrate to be used may be judged by a rough titration into 2 c.c. of the dye.

The reliability of the indophenol dye-ascorbic acid reaction and recoveries obtained by its use have been amply shown.^{1, 2} It was necessary only to check on the quantitative extraction of the oxidized dye from acid solution by xylene and the applicability of Beer's law to the xylene solution of dye. Both of these stipulations were satisfied in the concentration range of dye utilized in this test. Table I illustrates the agreement of the values found on several samples of blood by Bessey's method² and the present modification.

REFERENCES

1. Mindlin, R. L., and Butler, A. M.: The Determination of Ascorbic Acid in Plasma; A Macromethod and Micromethod, *J. Biol. Chem.* 122: 673, 1938.
2. Bessey, O. A.: A Method for the Determination of Small Quantities of Ascorbic Acid and Dehydroascorbic Acid in Turbid and Colored Solutions in the Presence of Other Reducing Substances, *J. Biol. Chem.* 126: 771, 1938.
3. Bukatsch, F.: A New Method for the Determination of Vitamin C With the Zeiss Step-Photometer, *Ztschr. physiol. Chem.* 262: 20, 1939.

A SIMPLE RAPID METHOD FOR DETERMINING RELATIVE BLOOD VOLUME CHANGES BY SPECIFIC GRAVITY STUDIES*

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RECENT advances in the study and treatment of shock have made necessary a simple practical method for determining changes in blood volume during the development and treatment of that condition. We present herein a method which is thought to represent such a procedure based entirely on specific gravity determinations.

Methods that are at present in use for estimating blood volume changes uniformly depend upon the determination of the concentration of various substances in the circulating blood, and from the known total amount of that particular substance in the blood, calculating the total volume of blood therefrom. With this general principle as a common feature there are two separate, practical approaches that may be utilized. The first of these is the dye method, first introduced in 1915 by Keith, Rowntree, and Geraghty.¹ These investigators determined the blood volume from the concentration of vital red injected into the circulating blood. The most significant modification of this method has been that of Gregersen, Gibson, and Stead,² who introduced Evan's blue as the dye and determined the dye concentration by photoelectric colorimetry. Although there are disadvantages and sources of error in these methods, their use has become well established in recent years. It is, however, with the second of these general methods for determining blood volume changes that the present consideration is particularly concerned. This method takes into account the changes in concentration of red blood cells, hemoglobin, or plasma proteins. Kottman³ in 1906 was the first to utilize this principle. In more recent years a number of investigators have studied blood volume changes, particularly after injection of isotonic and hypertonic fluids, using hemoglobin changes as the basis of blood volume alternations. Most of these workers have used difficult gasometric methods for hemoglobin determination. Bogert, Underhill, and Mendel⁴ showed that the variations in hemoglobin were an accurate indication of relative blood volume change by simultaneous determinations of total solids of the blood. Smith and Mendel,⁵ Blalock, Beard, and Thuss,⁶ Miller and Poindexter,⁷ and Robertson⁸ obtained apparently good results in estimating

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blood volume change by hemoglobin alterations after intravenous injections of fluids in animals. Gilligan, Altschule, and Volk⁹ used changes in the hematocrit to calculate changes in blood volume in human beings following intravenous injections of various fluids. These investigators were able to compare the results of this method with the dye method of Gregersen, Gibson, and Stead² in a few cases. They found the results to be in good accord.

Investigators, particularly Lamson and Rosenthal¹⁰ and Miller and Poin-dexter,⁷ have seriously objected to the older dye methods on the basis of inaccuracy. However, these workers failed to take into account the residual dye remaining from previous injections in repeated blood volume determinations. This source of error has been clarified by Smith.¹¹ The method of Gregersen, Gibson, and Stead² at present must be considered the most accurate means of determining a given blood volume level, but is not itself free from disadvantages and sources of error. Particularly in cases of shock and burns under clinical and experimental conditions, the curve of disappearance of the blue dye will be variable because of variable degrees of capillary damage. Under these same circumstances there will be relatively less disappearance of red blood cells from the circulating blood. Furthermore, in the use of the dye method for repeated blood volume estimations, the necessity of determining the disappearance curve in a particular subject is an almost insurmountable disadvantage in clinical shock and burns; to a less extent is this true under experimental conditions.

In studying the alterations of the blood in experimental shock in dogs, attention was called to the great practical value of estimating changes in blood volume by changes in the hematocrit. It was also found that the red blood cell percentage (hematocrit) could be accurately determined from the specific gravity of whole blood and plasma. This report is, therefore, designed to illustrate the simple and practical manner of determining relative blood volume changes from specific gravity determinations. The method is adapted only to the determination of approximate plasma volume changes, and not to the accurate determination of the blood volume level. The procedure is based on calculating the hematocrit from the specific gravity of whole blood and plasma, and determining from changes in the hematocrit, alterations in the plasma volume.

METHODS

Dogs were used in all experimental work. Anesthesia was accomplished by intravenous injection of nembutal. Blood samples were taken at varying intervals, an average of four samples for each experiment. Blood was withdrawn from exposed jugular veins so that no compression of the vein was necessary. Shock was produced by trauma to extremities, trauma to intestine, intraperitoneal injection of 25 per cent sodium chloride, or application of tourniquets to both legs with subsequent release of the tourniquets. The average duration of the experiments was nine hours. Simultaneously blood pressure was determined by mercury manometer connected with the carotid artery. Heparin was used as the anticoagulant. On all samples of blood red cell counts, hematocrits, specific gravity of whole blood, and specific gravity of the plasma were determined. The hematocrit was determined according to the method of Win-

trobe,¹² centrifugation being carried out for forty-five minutes at 3,000 r.p.m. The specific gravity of whole blood and plasma were determined by the Falling Drop Method of Barbour and Hamilton.¹³

PROCEDURE

The hematocrit may be determined by specific gravity determinations of blood if it is recognized that the specific gravity of the whole blood is dependent upon the specific gravity of the red blood cells, the specific gravity of the plasma, and the proportions of the latter two components. This fact is apparent since the specific gravity of a mixture of two solutions is proportionately dependent upon the specific gravities of the two solutions. Assuming that solution A is composed of 4 c.c. of solution B and 2 c.c. of solution C, then

$$(1) \text{ Sp. Gr. of A} = \frac{(\text{Sp. Gr. of B} \times 4) + (\text{Sp. Gr. of C} \times 2)}{6}$$

The same will be true of whole blood, as follows:

$$(2) \text{ Sp. Gr. of Whole Blood} = \frac{(\text{Sp. Gr. of R.B.C.} \times \text{R.B.C. } \%) + (\text{Sp. Gr. of Plasma} \times 100 - \text{R.B.C. } \%)}{100}$$

Solving for red blood cell percentage,

$$(3) \text{ R.B.C. } \% (\text{Hematocrit}) = \frac{(\text{Sp. Gr. of Whole Blood} \times 100) - (\text{Sp. Gr. of Plasma} \times 100)}{\text{Sp. Gr. of R.B.C.} - \text{Sp. Gr. of Plasma}}$$

It is at once obvious that for the hematocrit to be determined from equation (3), the specific gravity of whole blood, of plasma, and of the red blood cells must be known. The latter factor should be a constant, and the former two factors should be readily determinable by the Falling Drop Method. It is an easy matter to ascertain the specific gravity of red blood cells from equation (2) if the specific gravity of whole blood, specific gravity of plasma, and hematocrit are known:

$$(4) \text{ Sp. Gr. of R.B.C.} = \frac{(\text{Sp. Gr. of Whole Blood} \times 100) - (\text{Sp. Gr. of Plasma} \times 100 - \text{Hematocrit})}{\text{Hematocrit}}$$

From a series of 32 blood samples shown in Table I, taken from ten dogs selected at random from a larger series, it has been possible to determine the mean specific gravity of red blood cells to be 1.0953, with a standard deviation of 0.00205. Substituting then, 1.0953 in equation (3), it is possible to solve for the hematocrit if the specific gravity of whole blood and specific gravity of plasma are known. Table I shows that the red blood cell percentage calculated by this means differs only slightly from the actual hematocrit. To facilitate the determination of the hematocrit from the specific gravity of the whole blood and of the plasma, a nomogram, Fig. 1, has been constructed.

In using the hematocrit to estimate blood volume changes, it is necessary to determine an original blood volume by the dye method or to assume an original blood volume based on body weight. Blood volume in dogs has been estimated as 0.0915 of body weight. The total volume of red blood cells can then be determined from the hematocrit and the original blood volume:

Total R.B.C. Volume = Blood Volume (0.0915 of Body Wt.) \times Hematocrit

Assuming this total volume of red blood cells to remain constant, the resulting

blood volume can then be calculated from the subsequent hematocrits as follows:

$$\text{Blood Volume (2)} = \frac{\text{Red Blood Cell Volume} \times 100}{\text{Hematocrit (2)}}$$

In order to simplify this estimation Fig. 2 represents a nomogram which may be used to determine quickly the blood volume resulting after change in the hematocrit.

TABLE I

SPECIFIC GRAVITY OF RED BLOOD CELLS AND RED BLOOD CELL PERCENTAGE CALCULATED FROM SPECIFIC GRAVITY OF WHOLE BLOOD AND PLASMA

DOG NO.	BLOOD SP. GR.	PLASMA SP. GR.	HEMATO-CRIT	HEMATO-CRIT CALCULATED	ERROR	ERROR PER CENT	R.B.C.* SP. GR.	REMARKS
5	1.0511	1.0248	36	37	1.0	2.6	1.0978	Leg traumatized but dog did not go into shock
	1.0525	1.0248	37.5	39	1.5	4.0	1.0989	
	1.0547	1.0274	39.5	40	0.5	1.3	1.0965	
	1.0552	1.0257	41	42	1.0	2.4	1.0976	
	1.0582	1.0275	45	45.5	0.5	1.1	1.0964	
11	1.0542	1.0231	42.5	43	0.5	1.2	1.0963	Control dog
12	1.0511	1.0250	37	37	0	0	1.0955	Leg traumatized; dog died from shock
	1.0526	1.0261	39	38.3	0.7	1.8	1.0938	
	1.0596	1.0276	47	47	0	0	1.0956	
	1.0615	1.0281	49	49	0	0	1.0962	
	1.0661	1.0286	53.5	55.7	2.2	4.1	1.0986	
14a	1.0479	1.0234	34.5	33	1.5	4.3	1.0944	Shock by manipulation of intestines
	1.0604	1.0253	50	50	0	0	1.0955	
16	1.0497	1.0212	39.7	38	1.7	4.3	1.0930	Shock not produced by trauma to intestines
	1.0487	1.0222	37	36	1.0	2.5	1.0938	
	1.0501	1.0228	37.7	38	0.3	0.8	1.0949	
	1.0531	1.0235	39.7	41	1.3	3.5	1.0981	
	1.0537	1.0239	42	42	0	0	1.0948	
18	1.0488	1.0215	34.9	36.5	1.6	4.6	1.0940	Dog shocked by trauma to intestines
	1.0521	1.0221	40.2	41	0.8	2.0	1.0967	
	1.0512	1.0225	37.7	39	1.3	3.5	1.0986	
	1.0635	1.0252	53.5	54	0.5	0.9	1.0968	
17	1.0470	1.0181	37.8	37	0.8	2.1	1.0945	Dog died from nem-butal
	1.0531	1.0200	43.5	44	0.5	1.1	1.0963	
20	1.0450	1.0211	33.5	32	1.5	4.5	1.0924	Shocked by sodium chloride intraperitoneally
	1.0514	1.0220	39	40	1.0	2.6	1.0974	
23	1.0418	1.0240	26.6	24	2.6	9.8	1.0910	Shock by tourniquet to legs
	1.0535	1.0278	40	38	2.0	5.0	1.0920	
	1.0583	1.0281	45.8	45	0.8	1.7	1.0946	
26	1.0519	1.0233	42	39.7	2.3	5.5	1.0914	Shock by tourniquet to legs
	1.0567	1.0265	46	44	2.0	4.3	1.0920	
	1.0729	1.0311	67	64	3.0	4.5	1.0935	

*Mean specific gravity of red blood cells—1.0953.

Standard deviation—0.0020 or 0.2053 per cent.

RESULTS

Using the principles indicated above, it is possible to determine relative blood volume changes in a time as short as ten minutes, most of which time required for a short centrifugation of the blood sample in order that a small amount of plasma may be obtained for specific gravity determination. In order to illustrate the manner of application and the relative accuracy of the present method, data on six dogs selected from a larger series are presented in Table II. It will be seen from the table that there is a close correlation between

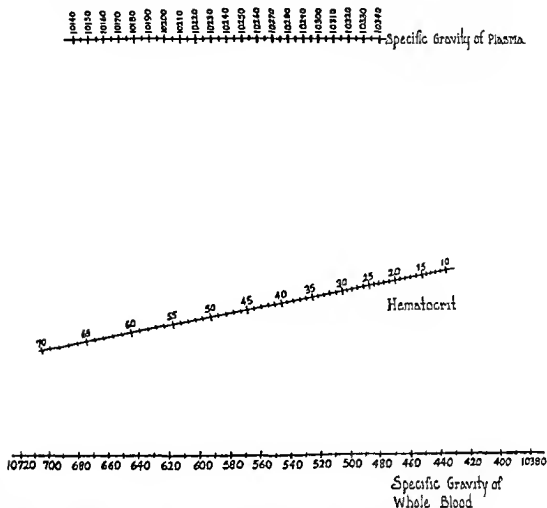


Fig. 1.—Nomogram for determining hematocrit from specific gravity of whole blood and plasma. A straight line from the specific gravity of whole blood and plasma crosses the middle line at the hematocrit level.

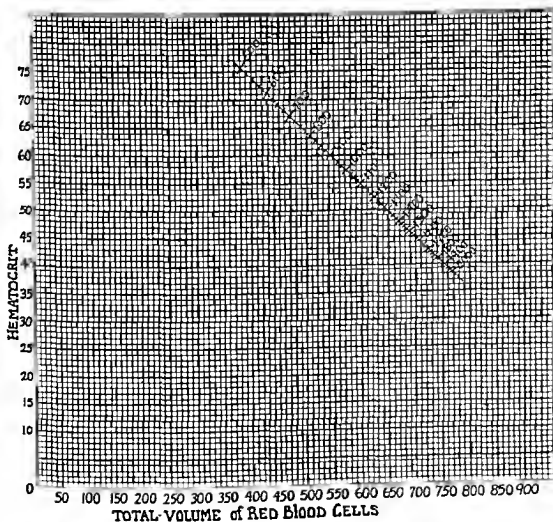


Fig. 2.—Nomogram for determining the blood volume changes from the hematocrit. A straight line from point 0 to the blood volume crosses the horizontal line of the original hematocrit at a point where the latter is intersected by the vertical line of the red blood cell volume. Then a line drawn from 0 through the point of intersection of red blood cell volume and subsequent hematocrits, crosses the blood volume indicator at the point of resulting blood volume.

TABLE II

BLOOD VOLUME CHANGES IN EXPERIMENTAL SHOCK AS CALCULATED FROM SPECIFIC GRAVITY OF WHOLE BLOOD AND PLASMA

DOG NO.	BLOOD SP. GR.	PLASMA SP. GR.	HEMATO-CRIT	R.B.C. % CALCULATED	BLOOD VOLUME	MAXIMUM DECREASE OF BLOOD VOLUME	EXPECTED DECREASE (3% BODY WEIGHT)	REMARKS
12	1.0511	1.0250	37	37	830*	270	273	Shock by trauma to legs
Wt.	1.0526	1.0261	39	38.3	800			
9.1	1.0596	1.0276	47	47	660			
kg.	1.0615	1.0281	49	49	635			
	1.0661	1.0286	53.5	55.7	560			
15	1.0517	1.0231	38.7	39	1140*	405	375	Shock by trauma to intestines
Wt.	1.0553	1.0244	43	43.5	1025			
12½	1.0634	1.0271	52	52.5	840			
kg.	1.0688	1.0276	59	60.5	735			
19	1.0538	1.0241	41	41.5	915*	435	(720-250)	250 c.c. of 25% sodium chloride intraperitoneally. 720 c.c. fluid in peritoneum at autopsy
Wt.	1.0619	1.0240	49	52.5	720		470	
10	1.0741	1.0258	65	68	480			
kg.								
20	1.0451	1.0211	33.5	32	830*	355	(600-225)	225 c.c. of 25% sodium chloride intraperitoneally. 600 c.c. of fluid in peritoneum at autopsy
Wt.	1.0514	1.0220	39	40	660		375	
9.1	1.0596	1.0226	48.6	50	520			
kg.	1.0654	1.0265	53.9	55.5	475			
21	1.0517	1.0215	39	40	1080*	410	(700-250)	250 c.c. of 25% sodium chloride intraperitoneally. 700 c.c. in peritoneum at autopsy
Wt.	1.0655	1.0220	56	57.5	755		450	
12	1.0714	1.0248	68	65	670			
kg.								
25	1.0547	1.0266	41.2	41	710*	220	230	Shock by tourniquets to legs for 4½ hours
Wt.	1.0694	1.0298	61	60	490			
7.7	1.0693	1.0294	60.5	60	490			
kg.								

*Blood volume calculated as 0.0915 of body weight.

the actual hematocrit and the calculated hematocrit. In almost all instances the difference is not greater than the error to be expected in hematocrit determinations. Since in shock the degree of capillary damage is such that colloidal dyes escape irregularly from the circulating blood, it is not possible to check the results of this method by the usual dye methods of blood volume determination. Magladery, Solandt, and Best¹⁴ have recently pointed out the inadequacy of dye methods for blood volume determination in shock. It will, however, be noted that there is close agreement between the blood volume decrease based on these hematocrit values and the blood volume decrease expected to occur in the development of shock. This expected decrease is based on the body weight of the animals, 3 per cent of body weight or one-third of blood volume being expected to be lost when the duration of the experiment is as long as eight hours.¹⁵ The closest possible check on the method is afforded by the intraperitoneal injection of 25 per cent sodium chloride solution. Presumably all the lost plasma will be in the peritoneal cavity where it may be measured. In three dogs shown, Nos. 19, 20, and 21, it will be seen that there is accurate correlation in this regard.

DISCUSSION

In all circumstances where blood volume alteration due to increase or decrease of plasma volume occurs, these changes may be simply and accurately determined from the specific gravity of whole blood and plasma. Intermediately the hematocrit is calculated. Naturally, in those conditions where blood volume decrease is attended by an appreciable loss of red blood cells, the method will be of considerably less value. It should be remembered, however, that in those conditions where there is loss of red blood cells, the dye method will also be in error because of dye being lost from the blood stream. It would seem that wherever repeated estimations of blood volume may be accomplished by the indirect dye method, the relative blood volume changes may be computed from the specific gravity.

In experimental shock due to mild trauma to extremities, application of tourniquets, trauma to intestine, or intraperitoneal injection of hypertonic sodium chloride solutions, the method is particularly applicable. This is true because the loss of red blood cells is negligible under these circumstances.

In human beings the same methods should be applicable for determining relative blood volume changes. In a series of determinations of specific gravities of human red blood cells somewhat lower values than those for dogs have been obtained. The mean specific gravity of human red blood cells was found to be 1.0913, with a standard deviation of 0.0016. The formula for determining the red blood cell percentage (hematocrit) in man would then be:

$$\text{Hematocrit} = \frac{(\text{Sp. Gr. of Whole Blood} \times 100) - (\text{Sp. Gr. of Plasma} \cdot 100)}{1.0913 - (\text{Sp. Gr. of Plasma})}$$

Clinically, relative blood volume changes calculated from specific gravity of whole blood and plasma might be expected to be accurate in a great many circumstances. In cases of burn, where the decreased blood volume is due entirely to escape of plasma, the method should be particularly useful. This is especially true since most all cases of burn are seen quickly enough in hospitals that a sample of blood drawn on admission will reflect well the total red blood cell volume as calculated from the total blood volume determined from weight, and the red blood cell percentage determined from specific gravity of whole blood and of plasma. In the usual case of shock from trauma, operation, and anesthesia, intestinal obstruction, marked diarrhea, protracted vomiting, etc., as long as there is no excessive loss of blood, the method should be suitable. Where blood volume changes temporarily occur from injection of various isotonic and hypertonic solutions as well as whole or concentrated plasma, it will be very simple to determine these changes by changes in the specific gravity.

It should be pointed out that the specific gravity of the red blood cells of dogs and of human beings varies not only in different individuals but also in the same individual at different times. The degree of variation, however, which has so far been encountered, has not been sufficient to cause an appreciable error in the calculations. A subsequent report shall consider in greater detail the specific gravity of human red blood cells and the variation of this property under different circumstances.

SUMMARY

1. The hematocrit, or red blood cell percentage changes reflect accurately the plasma volume changes under circumstances where there is no appreciable loss of red blood cells.

2. A new, quick method for determining red blood cell percentage by specific gravity studies of whole blood and plasma is presented.

3. Blood volume changes in experimental shock due to mild trauma, intraperitoneal injections of hypertonic solutions of sodium chloride, or application and subsequent release of tourniquets may be accurately followed by this method.

4. Clinically the method should prove of value in studying the relative volume changes in shock due to trauma, operations and anesthesia, intestinal obstruction, severe diarrhea, and protracted vomiting. The same procedure should be an accurate guide to fluid therapy in shock and other conditions.

REFERENCES

1. Keith, N. M., Rowntree, L. G., and Geraghty, J. T.: A Method for the Determination of Plasma and Blood Volume, *Arch. Int. Med.* 16: 547, 1915.
2. Gregersen, M. I., Gibson, J. J., and Stead, E. A.: Plasma Volume Determination With Dyes, Errors in Colorimetry; Use of the Blue Dye T—1824, *Am. J. Physiol. (Proc.)* 113: 54, 1935.
3. Kottman, K.: Ueber die Bestimmung der Blutmenge beim Menschen und Tier unter Anwendung eines neuen Präzisionshämatocriten, *Arch. f. exper. Path. u. Pharmacol.* 54: 536, 1906.
4. Bogert, L. J., Underhill, F. P., and Mendel, L. B.: The Regulation of Blood Volume After Injections of Saline Solutions, *Am. J. Physiol.* 41: 189, 1916.
5. Smith, A. F., and Mendel, L. B.: The Adjustment of Blood Volume After Injection of Isotonic Solutions of Varied Composition, *Am. J. Physiol.* 53: 323, 1920.
6. Blalock, A., Beard, J. W., and Thuss, Charles: Intravenous Injections. A Study of the Effects on the Composition of the Blood of the Injection of Various Fluids Into Dogs With Normal and Low Blood Pressures, *J. Clin. Investigation* 11: 267, 1932.
7. Miller, J. R., and Poindexter, C. A.: The Effects Observed Following the Intravenous and Subcutaneous Administration of Fluid. An Experimental Study on Dogs, *J. LAB. & CLIN. MED.* 18: 287, 1932.
8. Robertson, J. D.: The Effect of Hemorrhage of Varying Degree on Blood and Plasma Volume, on Blood Sugar, and on Arterial Blood Pressure, *J. Physiol.* 84: 393, 1935.
9. Gilligan, D. R., Altschule, M. D., and Volk, M. C.: The Effects on the Cardiovascular System of Fluids Administered Intravenously in Man. I. Studies on the Amount and Duration of Changes in the Blood Volume, *J. Clin. Investigation* 17: 7, 1938.
10. Lamson, P. D., and Rosenthal, S. M.: The Inadequacy of Our Present Blood Volume Methods, *Am. J. Physiol.* 63: 358, 1923.
11. Smith, H. P.: Intravenous Injections of Fluid and Repeated Blood Volume Determination, *Bull. Johns Hopkins Hosp.* 37: 177, 1925.
12. Wintrobe, M. M.: A Simple and Accurate Hematocrit, *J. LAB. & CLIN. MED.* 15: 257, 1929.
13. Barbour, H. G., and Hamilton, W. F.: The Falling Drop Method for Determining Specific Gravity, *J. Biol. Chem.* 69: 625, 1926.
14. Magladery, J. W., Solandt, D. Y., and Best, C. H.: Serum and Plasma in the Treatment of Haemorrhage in Experimental Animals, *Brit. M. J.*, Aug. 24, p. 248, 1940.
15. Blalock, Alfred: Experimental Shock. VI. The Probable Cause of the Reduction in the Blood Pressure Following Mild Trauma to an Extremity, *Arch. Surg.* 22: 598, 1931.

DEPARTMENT OF ABSTRACTS AND REVIEWS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

LEVULOSURIA, Study of Two Cases in Brothers, Jacobsen, V. C. *Am. J. M. Sc.* 200: 304, 1940.

Two cases are reported of so-called essential levulosuria in young Jewish brothers. They bring the total on record to 33. The condition appears to be a metabolic anomaly in which the organism exhibits a partial or complete inability to convert levulose into glycogen. This seems to be due not to a hepatic deficiency, but to increased permeability of the kidneys by the levulose molecule. This renal hypothesis does not postulate any inherent kidney disease, although in one case with paroxysmal hypertension there were signs of renal irritation. In both cases an approximately equal amount of glucose was excreted, but only at the same time that levulose was passing through the kidneys. Normal dextrose and levulose tolerance tests were obtained.

The clinical importance of the condition lies in its early recognition, before the patient is subjected to the rigors of a diabetic regimen which may severely handicap the growing child. The treatment is reassurance that he does not have diabetes mellitus, and a diet without special restrictions. So far as is known, the passage of levulose through the kidneys causes no harm, although continual observation of these interesting people throughout their lives may later alter this dictum in some degree.

SERUM, Significance of Albumin-Globulin Ratio of, Melnick, D., Field, H., Jr., and Farnall, C. G., Jr. *Arch. Int. Med.* 66: 295, 1940

The albumin-globulin ratios characteristic of normal and hypoproteineamic sera represent the relative amounts of two independent protein systems, which may be separated by salting-out procedures. Dissociation and association phenomena attributed to the serum protein molecules must occur within these independent systems. Exposing normal and hypoproteineamic sera to the same environmental factors by dialysis of one against the other does not alter the ratio of one protein fraction to another. The influence of incipient denaturation on the salting-out behavior of the serum protein complex has been studied and serves to explain the alleged conversion of albumin to globulin reported by others. Urinary protein does not represent total serum but is derived principally from the albumin fraction.

The inadequacy of colloid osmotic pressure measurements *in vitro* and of the determinations of albumin and globulin for predicting the true oncotic pressure *in vivo* are discussed in the light of recent physicochemical studies of the serum protein complex.

PNEUMOCOCCI, Immune Reactions of Carriers and Non-Carriers of Type-Specific, Finland, M., Brown, J. W., and Barnes, M. W. *Am. J. Hyg.* 32: 24, 1940.

Cultural and serologic studies were carried out in a selected group of the personnel of the medical wards of a general hospital in which large numbers of patients with pneumonia are admitted for treatment. The studies were directed primarily to the isolation and identification of pneumococci and their types and the determination of type-specific pneumococcus antibodies.

The most efficient method found for detecting pneumococcus carriers consists of inoculating a swab of the pharynx into suitable culture media, injecting some of the growth resulting after four to six hours' incubation into a mouse and then examining the peritoneal washings after six to twenty-four hours.

Fifty-six per cent of the persons studied were found to harbor pneumococci in their pharynx on one or more occasions. The percentage of carriers detected was related to the

number of times the cultures were made. However, those in whom pneumococci were not found in the first culture were less likely to have them in subsequent cultures and, if pneumococci were acquired, they were more readily lost. The duration of the carrier state varied considerably.

The finding of pneumococci during this study was apparently unaffected by the recent presence of acute respiratory infections.

The distribution of pneumococcus types encountered showed a predominance of some types, such as III, VI, and XIII, which are usually frequent among normal healthy carriers. Twenty-six different specific types were encountered, and only 1 per cent of the strains of pneumococci failed to react with serums for types I to XXXII. In some instances mouse passage was necessary before the type could be determined.

Carriers of pneumococci frequently had, or acquired, homologous type-specific antibodies. The time of appearance of such antibodies in relation to the finding of the pneumococci, and the length of time over which the antibodies could be detected varied widely and was not always related to the duration of the carrier state.

Some indirect evidence was adduced which indicated that certain of the personnel acquired some of their pneumococci from patients under their care. In many instances, however, the findings suggested that the carriers acquired the various pneumococci from one another.

Four of the patients developed atypical pneumonia in the course of this study. Two of these were known to be chronic carriers, and the same types were found during their illness; one had a few pneumococci in his sputum during his illness but none before or later; and the fourth was a transient carrier before the onset of his pneumonia, but no pneumococci could be found during his illness. None of these four patients developed significant antibodies either to the pneumococcus which was found or to most of the common types associated with pneumonia.

These findings lend further support to the antigenicity of type-specific pneumococci in apparently healthy carriers.

The findings in the patients who developed pneumonia indicate that pneumococci found in patients with atypical pneumonias may not be etiologically related to the infection.

TISSUE: Improved Method of Staining Within Tissues, Leptotriches of Parinaud's Conjunctivitis and Gram-Positive Micro-Organisms, Verhoeff, F. H. J. A. M. A. 11 1546, 1940.

Fix fresh tissue in Zenker's solution or solution of formaldehyde.

Embed in celloidin or paraffin. Celloidin sections are the more readily manipulated.

Cut sections as thin as possible, not more than 10 microns in thickness.

Stain sections in hematoxylin and eosin.

Mount, examine, and select sections that contain the largest foci of macrophages.

By the aid of xylene remove the selected sections from the slide and wash in oil of thyme, then in 95 per cent alcohol, and then in water. (Hematoxylin may be removed by acid alcohol, but this is not necessary.) If large foci of macrophages have been found, adjacent unstained sections will undoubtedly also contain them and may, therefore, be used instead.

The solvents now to be used are placed in small receptacles, such as ordinary salt cellars. Celloidin sections are to be immersed in these. In the case of paraffin sections the solutions are to be dropped on the slides by means of eye droppers. Water is placed in large finger bowls.

Place the celloidin section from the water on a slide. Wipe away any excess of water and drop on the section Stirling's crystal violet (methyl rosaniline 5 Gm., 95 per cent ethyl alcohol 10 c.c., aniline 2 c.c., water 88 c.c.) for two minutes.

Float the section from the slide into a bowl of water. Wash in several changes of water; this requires about two minutes.

Immerse in compound solution of iodine, from twenty to sixty seconds (iodine 1 Gm., potassium iodide 2 Gm., water 100 c.c.).

Wash in water and transfer to 95 per cent alcohol. Stir the section around until the stain begins to come out freely, about five seconds.

Transfer quickly to trichlorethylene. Stir the section around until the color ceases to come out freely, about ten seconds.

Transfer to oil of thyme one minute, then to 95 per cent alcohol for five seconds, and again to oil of thyme. Examine on the slide under the low power of the microscope. If precipitates are present, or differentiation is insufficient, differentiate further by alternate immersions in 95 per cent alcohol and oil of thyme, five seconds each.

Wash in xylene, two changes, and mount in cedar oil on a slide.

By this method the leptotriches should be deeply stained and easily recognizable when occurring, as is usually the case, in masses. Isolated leptotriches will probably not be recognized by inexperienced observers. If no leptotriches are visible, other structures, such as fibrin, granules of mast cells, and chromatin, may possibly be mistaken for them by those who have never seen these microorganisms. When leptotriches are present in masses, however, their appearance is so characteristic and so different from any other structures in the tissues that they are easily identified.

PNEUMONIA, The Sabin Agglutination Test and the Polysaccharide Skin Test (Francis)
as Indices of Recovery in, Fox, W. W., Rosi, R., and Winters, W. L. *Am J. M. Sc.*
200: 649, 1940.

The authors conclude from their observations that the agglutinin test is a reliable index to the development of increased resistance to the pneumococcus in the course of pneumonia. It is such a simple test that it may be readily used as a control for sulfapyridine treatment. It rarely becomes positive before the eighth day of the disease; and since in complicated cases it is delayed until the twelfth to thirteenth day, it is particularly important that sulfapyridine be continued longer in such cases. Relapse and late septic complications may be prevented if this is done.

The Francis test is reliable in only 50 per cent of the cases (with the polysaccharide solutions used). The solutions of polysaccharide are available only for the more common types.

A change from negative to positive Francis test occurs on an average of two days before the agglutinin test becomes strongly positive. When this change is noted, it is probably safe to decrease the dosage of sulfapyridine, and to discontinue it two days later.

As concluded in a previous paper, recovery in pneumonia treated with sulfapyridine depends on three factors: an adequate early leucocytosis, an adequately maintained sulfapyridine blood level, and development of active immunity by the patient for the type of pneumococcus causing his pneumonia. The agglutinin test is a simple, inexpensive, rapid method for determining the development of active immunity.

INTOXICATION, Use of the Urine in the Chemical Test for, Haggard, H. W., Greenberg, L. A., and Miller, R. P. *J. A. M. A.* 115: 1680, 1940.

In the medicolegal diagnosis of intoxication the concentration of alcohol in the blood is frequently estimated indirectly from that found in the urine.

Unless special precautions are taken, serious errors may occasionally occur.

The ratio of the concentration of alcohol in blood to that of urine varies only slightly with the specific gravity of the urine; the ratio as determined is 1:1.3.

Absorption of alcohol from the bladder into the blood does not occur to a significant extent if the concentration found in the urine after drinking alcohol.

PREGNANDIOL EXCRETION, Diagnostic Value of, in Pregnancy Disorders, Cope, C. L. *Brit. M. J.* 2: 545, 1940.

Pregnanediol is a product of corpus luteum and placental metabolism. Its excretion in the urine is relatively easily measured in any biochemical laboratory. An attempt has been

made to assess the diagnostic value of pregnanediol excretion in abnormal pregnancy. Its complete absence is nearly always evidence of serious abnormality, suggesting in early pregnancy the death of the fetus. The significance of a low pregnanediol excretion still remains uncertain. A normal pregnanediol excretion is not evidence that abortion will not occur or that the fetus is still alive. Both chronic nephritis and toxemia of pregnancy may interfere with pregnanediol excretion. Deviations from the normal excretion in these conditions do not necessarily mean that the gestation is abnormal. Pregnanediol excretion seems assured of a place as a diagnostic aid in obstetric disorders.

BLOOD COAGULATION, Effect of Nicotinic Acid on, Calder, R. M., and Kerby, G. P.
Am. J. M. Sc. 200: 590, 1940.

Clinical experience in a significantly large group of patients with chronic brucellosis revealed that nicotinic acid possesses the property of promoting coagulation of the blood. These patients often develop subcutaneous hemorrhages after slight trauma, and their blood clots slowly, retraction is imperfect, and the yield of serum is low. All these defects were completely remedied by the administration of nicotinic acid.

These observations were extended to a small group of patients with other clinical states associated with bleeding, and the results were uniformly encouraging. Presumably, the hemorrhagic tendencies in all these patients rested on a toxic, infectious basis.

Experimental studies reported show that nicotinic acid does not duplicate the action of, nor can it be substituted for, any of the known factors involved in blood clotting (calcium, platelets, thromboplastic principle, prothrombin, or thrombin). However, when it is added in vitro to blood containing excessive amounts of antithrombin (heparinized blood or samples from dogs in peptone or anaphylactic shock), it does induce coagulation. That this effect is not due to nonspecific alteration of hydrogen-ion concentration was indicated by contrast of nicotinic acid with seven other acids, none of which was as active in this respect as was nicotinic acid.

The quantities of nicotinic acid employed in in vitro experiments were so enormous when compared with the doses used clinically, that it is not justifiable to assume that the mechanism of its effect in patients is necessarily a chemical neutralization of antithrombin. Though such an explanation is possible. Certainly, in view of the small doses effective in patients, the results cannot be attributed to a nonspecific acid effect. It would seem logical to conclude that nicotinic acid corrects a fundamental deficiency in the organism; or, by some pharmacodynamic action (e.g., stimulation of the liver), induces the formation either of more prothrombin or of less antithrombin.

Whatever the mechanism of its action may be, experience indicates that further trial of nicotinic acid in various hemorrhagic states is warranted.

PNEUMONIA, PNEUMOCOCCIC, Should Serum Be Used in Addition to Sulfapyridine?
Dowling, H. F., Abernethy, T. J., and Hartman, C. R. J. A. M. A. 115: 2125, 1940.

A series of cases of pneumonia caused by pneumococcus type I through type VIII have been alternated for treatment with sulfapyridine alone and with sulfapyridine plus specific antipneumococcus serum. The mortality rate was 12.5 per cent in the group receiving sulfapyridine alone, and 9.8 per cent in the group receiving serum plus sulfapyridine. If the type III cases are omitted, the corresponding figures are 9.2 and 4.5 per cent, respectively.

Serum seemed to be particularly valuable as an adjunct to sulfapyridine in patients over 40 years of age.

Crisis occurred more frequently and was more prompt in the patients receiving serum in addition to sulfapyridine.

It is suggested that serum and sulfapyridine both be given to patients over 40 years of age with pneumonia and to those who are in need of a prompt defervescence.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor,
Dr. Warren T. Vnughan, 201 West Franklin Street, Richmond, Va.

The Methods of Ferment Investigation* (Section 1)

THE Methods of Ferment Investigation is intended to review our present knowledge of all ferment methods used in industry, biology, and medicine. The complete work will have about 3,500 pages and will contain 214 different articles written by 131 collaborators from various countries throughout the world. The book will be written entirely in German. Up to now only the first section of the book has appeared. A short introduction given by E. Bamann (Tübingen) and K. Myrbäck (Stockholm). The first subdivision deals with the substrates of ester-splitting ferments: substrates of the lipases by E. Bamann, natural glycerides by K. H. Bauer (Leipzig), acetylcholine by R. Ammon (Königsberg), substrates of the tannases by O. T. Schmidt (Heidelberg), chlorophyll by H. Fischer (München), phosphoric acid esters by E. Hackenthal and M. Kobel (Berlin), phosphatides by S. Belfanti, A. Ercoli, and M. Francioli (Milan), phosphoric acid by T. Posternak (Genf), and sulfuric acid esters by E. Hackenthal and M. Kobel. The second subdivision discusses the carbohydrates and glycosides: sugar by E. L. Hirst (Bristol) and S. Peat (Birmingham), isolation of natural heterosides by J. Rabate (Paris), synthetic glycosides by H. Elsner (Berlin), and thioglycosides by F. Wrede (Berlin).

Despite the obvious fact that most of the authors tried to condense their material as far as possible, nearly all articles are written rather clearly and contain an enormous amount of useful literature references. For the moment, it is a disadvantage that all these references, which are cited only by name and number in the individual articles, are combined into one bibliography which will be published as the last section of the whole work.

—Ernst Fischer.

The Methods of Ferment Investigation† (Sections 2, 3, 4)

THE three recently published sections of the "Methods of Enzyme Investigations" contain more than 60 different articles by a large number of collaborators from various countries. The subdivision about carbohydrates and glycosides, started in the first section, is continued now by articles by A. Purr (Prag) about glycolysis and starch, by H. Elsner (Berlin) and co-workers about galactogen, fructosans, and chitin. Glucosans and zytans, ascorbic acid, and related substances are discussed by E. L. Hirst (Bristol) and S. Peat (Birmingham), polyuronides by H. Bock (Karlsruhe), chondroitin sulfuric acid by T. Soda (Tokyo), the phosphorus-free derivatives of the carbohydrates by H. K. Baerenscheen and J. Pany (Wien), and the phosphorus-containing derivatives by R. Robinson and M. G. Macfarlane (London). The next subdivision is formed by a lengthy contribution of W. Klein (Freiburg) about the nucleic acids and their derivatives and by some shorter chapters about the proteins and their derivatives by A. Schäffner (Prag), E. Abderhalden (Halle), and F. Leuthard (Zürich). The amides are discussed in an especially interesting article by C. G. Holmberg (Lund), the thiols and disulfides by T. Bersin (Marburg), and finally the nucleoproteins, lipoproteins, and glycoproteins by St. J. V. Przylecki (Warsaw).

The third section is mainly devoted to the description of the modern methods in enzyme investigations. K. Felix (Frankfurt) describes the use of enzymatic investigation for the determination of the chemical structure of substances of high molecular weight, while T. W.

*Die Methoden der Fermentforschung. Edited by Eugen Bamann, M.D. and Karl Myrbäck, M.D. Section 1 with 172 pages and 2 figures. Georg Thieme, Leipzig, 1940.

†Die Methoden der Fermentforschung. Edited by Eugen Bamann, M.D. and Karl Myrbäck, M.D. Section 2 with 303 pages and 6 figures. Section 3 with 392 pages and 249 figures. Section 4 with 407 pages and 177 figures. Georg Thieme, Leipzig, 1940.

Astbury (Leeds) and J. C. Derksen (Eindhoven) contributes three excellent chapters about x-ray spectrographic methods and their results. G. Kortüm and M. Kortüm-Seiler (Tübingen) discuss absorption spectrum, Raman spectrum, and fluorescence. Heyrovsky's polarographic methods for electrical potentials are described by R. Brdička (Prag), the magnetic methods by E. Müller (Jena), determination of viscosity by R. Mohr (Freiburg), and the ultracentrifuge and diffusion methods by O. Lamm (Uppsala). Short chapters about dielectric, kryoscopic, and osmotic methods for molecular weight determination are contributed by S. Arrhenius (Uppsala), D. Krüger (Berlin), and G. V. Schulz (Freiburg). L. Köfler (Innsbruck) describes microdetermination of the melting point and sublimation methods.

The rest of the third section and the whole fourth section deals with the enzymatic reactions themselves. E. Brunius (Stockholm) describes the various nomographic methods, M. Steiner (Göttingen) the maintenance and testing of suitable conditions for enzymatic reactions, and J. Lehman (Goteburg) the redox potential. "The calculation of the free energy of biochemical reaction" by W. Franke (München) is well written and instructive. The various physical and physico-chemical methods are summarized in several short chapters by L. Heilmeyer (Jena), B. J. Krijgsman (Utrecht), P. Wulff (Frankfurt), D. Krüger (Berlin), and by K. Linderstrøm-Lang and H. Holter (København), while the manometric methods are treated in a longer chapter by F. Dickens (Newcastle). The next subdivision is devoted strictly to chemical methods, especially micro-analytic methods: alcohols, aldehydes, and acids by M. Steiner and O. Glemser (Göttingen); carbohydrates by H. K. Barrensechen and J. Pany (Wien); colorimetric determination of carbohydrates by G. Haugard (København); purino bases by J. E. Jorpes (Stockholm); titration of the carboxyl and of the amino groups by W. Grassmann and P. Stadler (Dresden), and the volumetric and manometric determination of amino acids by the same authors. A short résumé of the more biological methods is given by E. Werle (Düsseldorf), while K. Linderstrøm-Lang and H. Holter (København) discuss very carefully the new histochemical methods. The last part of the fourth section contains the first chapters of the subdivision dealing with the isolation and testing of various enzymes. The general methods for preparation of enzyme-containing solutions are written up by H. Kraut and A. Weischer (Dortmund), the special methods for enzymes of invertebrates by B. J. Krijgsman (Utrecht), and those of bacteria by H. C. Werkman and H. G. Wood (Ames, Iowa), up to now the only American co-workers. Methods for the concentration of enzyme preparations by evaporation are summarized by L. Vogel and P. Laverenz (München), and separation methods by means of freezing are described by J. Hartmann (München). The two final chapters are very interesting reports by H. M. Karström (Helsinki) about bacteria as material for enzymatic investigations.

Without doubt, these three sections of the "methods of enzyme investigations" contain an enormous amount of useful material for investigation in this and related fields. It is regrettable that due to the war conditions, some of the non-German authors were unable to read the proofs, since otherwise some apparent errors due to the translation of their original articles would have been eliminated.

—Ernst Fischer.

Bacteriology of Public Health*

DIFFERING from other bacteriology books in that it does not incorporate laboratory methods, this text presents facts of pathologic bacteriology from a public health viewpoint. This has the effect of permitting the student to become oriented without being confused by too much unnecessary information; it does not, however, mean that the matter is dealt with in a superficial manner. Indeed, the author is to be commended on carefully weeding out that which is extraneous. The emphasis throughout is on the bacteriology of disease rather than on bacteria which incidentally cause disease. Following a historical review, one finds chapters on drinking water and disease, on food idiosyncrasies, food poisoning, and food infections, and then a chapter devoted to each of the bacterial, virus, and protozoal diseases. The book, which is well written, includes a good bibliography and index.

*The Bacteriology of Public Health. By George M. Cameron, Ph.D., Associate Professor of Bacteriology, University of Tennessee. Illustrated. 451 pages. The C. V. Mosby Company. St. Louis, 1940.

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PROGRESS

HEPARIN

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NATURE AND ORIGIN

THE discovery of heparin resulted from the finding of McLean¹ (1916), a pupil of Howell, that certain impure heart and liver phosphatides were inhibitory to blood coagulation. As the result of extensive investigations under the direction of Howell² (Johns Hopkins, Baltimore, 1916—), Best³ (Connaught Laboratories, Toronto, 1933—), Fischer⁴ (Carlsberg Foundation, Copenhagen, 1931—), Jorpes⁵ (Carolinian Institute, Stockholm, 1935—), the heparins have been separated from the phospholipids and identified as substituted polysaccharides belonging to the class of mucosaminopolysulfuric esters. The known molecular building stones are (1) hexuronic (? glyeauronic acid); (2) glucosamine, which is characteristic of the mucotins, just as galactosamine is typical of the chondroitins; (3) acetic acid (linked to the amino-N, in conformity with the rule that all amino sugars in nature are monoacetylated) (Furth and others, 1934); (4) several sulfuric acids (ester-linked to the hydroxyls of the hexuronic acid or hexosamine, or both). Many variations and fractionations are possible and purified natural products, including crystalline salts, differ in chemical details and in anticoagulant potency. According to the Toronto workers, there is a surprising similarity of the crystallization process for the various barium salts, and the varying potency does not parallel the sulfur content, as suggested by the Stockholm School. The simplest and most practical assay (Howell) defines a "unit" of heparin as the amount required to keep 1 c.c. of cat blood fluid for twenty-four hours at 0° C. The Toronto standard beef-lung heparin of Charles and Scott represents about 100 units per milligram. Potency and purity are not altogether synonymous when it comes to the question of toxic reactions.

Most tissues yield some heparin, but liver and lungs are the best sources in the majority of species. Holmgren and Wilander⁶ believe the origin to be in

the metachromatic granules, stainable with toluidine blue in the Ehrlich mast cells (tissue basophiles), which commonly cluster around the blood vessels in the tissues which are richest in heparin. The granules are depleted in peptone shock. The Toronto workers have also demonstrated a rise in blood heparin during serum anaphylaxis in dog experiments, and this rise, together with the associated incoagulability of the blood (but not the shock), could be prevented by excision of the liver, thus proving that the heparin comes from liver reserves.

BLOOD-CLOTTING MECHANISMS

Our experimentation⁶ (1934—) in the field of *blood coagulation* has led to the following conclusions as to the mechanisms involved: The fibrin of the clot is formed from the plasma protein, fibrinogen, through the agency of a coagulant, thrombin. The thrombin is first formed from an inactive precursor, prothrombin, which is also associated with the plasma globulins. Whether or not thrombin is an enzyme (fibrinogen denaturase, Wöhlisch,⁷ Fischer⁸) and prothrombin its zymogen (mother substance) is still unsettled. The coagulant is either a protein or very intimately associated with proteins throughout various phases of their colloidal behavior. Among enzyme analogies to thrombin may be mentioned the papainases. We have found a crystalline papain, kindly supplied by Dr. A. K. Balls, of the U. S. Department of Agriculture, clotted a prothrombin-free fibrinogen in twelve seconds, with lysis of the clot a few minutes later.

The conversion of prothrombin to thrombin requires certain activators, the chief one (we believe) being *thromboplastic enzyme*, a tryptase-like component of aqueous tissue extracts, which is also present, to some extent, in Berkefelded plasma and prothrombin made from it. Ionized calcium salts and phospholipid cephalin are essential cofactors. Much of our work has dealt with these thromboplastic agents.

The clotting reactions, especially in their time relations, are sensitive to physical conditions, including dilution, temperature, pH, salt content, "wetting" (lowering of surface tension), and other factors influencing colloidal stability. Specific inhibitors may be classified as (1) antiprothrombic (Howell) if they interfere with the first phase (conversion of prothrombin to thrombin), and (2) antithrombic if they affect the thrombin-fibrinogen interaction (second phase). "Immediate" antithrombins evince their full degree of inhibition at once. "Progressive" antithrombins, on the other hand, inactivate thrombin in a progressive manner, to which the term "thrombinolysis" is applicable, if it is demonstrably an enzymatic process. It is not convincingly proved that proteolytic phenomena in blood coagulation result in nonprotein nitrogen increase (as in ordinary protein digestion). Probably there is only a minor degradation and perhaps rearrangement of the significant protein complexes. The overt proteolytic enzyme, trypsin (e.g., Northrop and Kunitz's crystalline preparation) shows three actions, viz., (a) clot-aiding (thromboplastic), (b) thrombin-destroying (thrombinolytic), and (c) fibrin-dissolving (fibrinolytic). We have demonstrated parallel phenomena with aqueous tissue extracts (crude thromboplastins) and the first two, at least, can be shown with Berkefelded (i.e., cell- and platelet-free) plasma and serum and with the usual prothrombin

preparations. Clot retraction is absent or defective, and fibrinolysis is not seen in thromboplastin-poor plasmas and thrombins.

MODES OF ACTION OF HEPARIN

We have shown that heparin can inhibit the tryptic digestion of casein.⁸ It also retards the thrombin-destroying action of trypsin and of serum.⁹ In this, of course, it is antagonistic to progressive antithrombin, but the amounts of heparin needed are too large to convince us that this is more than a matter of theoretical interest.

In systems of purified clotting agents, heparin is practically devoid of antithrombic action, but the addition of a little plasma or serum results in the prompt appearance of a strong inhibition.¹⁰ Heparin *plus* a plasma cofactor (Howell's proantithrombin), therefore, forms an "immediate" antithrombin,¹¹ the actions of which are very similar to those of other immediate antithrombins, e.g., ferrocyanides (polyvalent anions). The plasma cofactor has not yet been defined, but we have definitely ruled out crystalline serum albumin.¹⁰

We have confirmed¹² another important action of heparin, namely, the antiprothrombic (Howell and Holt, 1918). Several recent authors deny the first phase action of heparin or postulate an essential cofactor on insufficient experimental grounds. The difficulty is due to their use of strong tissue thromboplastins which obscure the heparin effect. Our experiments¹³ clearly show that heparin has two distinct actions in the first phase of clotting. First, it retards the rate of prothrombin conversion to an extent which is inversely proportional to the amount of thromboplastin present. This fits in with thromboplastin studies¹⁴ and with the inhibitory effect of heparin on trypsin.⁸ It is concluded that heparin inhibits the enzyme factor in the thromboplastin and, if there is a small enough amount of enzyme, its prothrombin-activating function will be slow in consequence. Given an abundance of the thromboplastic enzyme, the retardation by heparin will be missed if it is not looked for in the first few minutes. Secondly, there is an effect on the amount (*effectiveness*) of the thrombin formed. This requires even more carefully chosen experimental conditions for its demonstration. With a strong prothrombin and small amounts of heparin, the alteration in final potency (thrombic) is negligible. But if the prothrombin is weak enough, an appreciable fraction is rendered ineffective and this can readily be demonstrated by suitable tests. Our recent fully-controlled tests demonstrate a first phase cofactor in crude plasma "albumin."¹³

A common basis for all heparin actions is suggested by Fischer's¹⁵ (1931) observation that it combines with proteins (and protamines, Chargaff¹⁶), thus altering their electrochemical and colloidal properties. Depending on the isoelectric points, this may apply alike to enzyme and substrate.

PRACTICAL APPLICATIONS OF HEPARIN

Clinical uses have increased with improvements in purification and consequent lessening of toxicity of heparin preparations. Howell tried out his best preparations in 1928 to 1930 and found them noninjurious to human beings. Mason¹⁷ and others, however, had very poor results with the commercial preparations. In 1935 to 1936 the Stockholm group⁵ (Sköld; Hedenius

and Wilander) used the purified preparations of Jorpes and Bergström with no untoward effects, and they have continued to recommend heparin for transfusion and thrombosis prevention. The Toronto group³ had been trying their numerous preparations on animals since 1932, and in 1937 Murray, Jaques, Perrett, and Best¹⁸ advocated the Charles and Scott product for human use in the prevention of thrombosis of veins following injury.

As with insulin, the purified drug is expensive, but it is now available from the Connaught Laboratories in reasonably priced 10 c.c. sterile ampoules, containing a stable saline solution, assaying 1,000 units per cubic centimeter. Orally, it is not effective. Subcutaneously, the action is prolonged (usually five to eight hours), but is uncertain in degree and there is danger of bleeding at the injection site. The route of choice, therefore, is the intravenous, with all the usual precautions. This is safe and the anticoagulant effect is optimal but somewhat transient (averaging sixty to ninety minutes). Repeated injections are less reliable and more uneconomical than the *continuous intravenous drip*, but even with this, there is likely to be much variation as to the effective dosage.²⁰ The Toronto workers³ have identified a heparinase which is said to be responsible for these variations. About 10 per cent is lost in the urine. It is best to keep the coagulation time at fifteen to twenty minutes (normal is five to seven minutes), and the heparinization must never be started until after the complete arrest of all hemorrhage. Postoperatively, the Toronto group¹⁸ wait for four to twenty-four hours, but the Scandinavians⁵ are somewhat less conservative (two to three hours; Crafoord). The duration of treatment must be dictated by experience, but is commonly one to two days. The onset of hemorrhage (of any degree) and of undue edema in cases with an overtaxed circulation calls for discontinuance of the treatment. Heparin does not soften clots but, as much by the preservation of the platelets and lessening of their agglutination as by its anticoagulant effects, it minimizes thrombus formation. When thrombi do occur, they absorb more readily and completely, with less danger of extension and of embolism. The protamine, *salmine-sulfuric acid*, is a physiologic antagonist to heparin both in vitro and in vivo.¹⁹ The action is quantitative¹⁶ and the protamine in excess is itself an anticoagulant. Being a foreign protein (split product), there is danger of allergy, but it could be tried in the event of dangerous hemorrhage in a heparinized patient.

The main field of clinical usefulness is in *prevention* of thrombosis and in lessening the dangers of embolism (Murray¹⁸). Vision has been restored in some cases of thrombosis of the central retinal vein, which, ordinarily, leads to almost certain blindness (Holmin and Ploman²¹). Besides cases of peripheral vessel, coronary, and cerebral thrombosis, there are reports on the use of heparin in endocarditis²² and in malaria.²³ The surgeon is especially interested in its use in traumatic and postoperative thrombosis. The prophylactic regime in these conditions has already been dealt with, but the necessity for choice of suitable cases may be re-emphasized.

Heparin is said to have revolutionized vascular surgery, including both anastomoses and operative removal of clots.¹⁸ The prospect of permanent patency of the sutured vessels is greatly increased. Good results have been reported in lessening the repetition of embolism in phlebitis cases.

Being poorly absorbed through serous membranes, heparin is suggested for use in the prevention of adhesions in serous cavities. Basic experimental work in this regard was performed in 1935 by Miki and Satani²⁴ (Kioto) on rabbits. The exposed omentum was scarified with dry gauze, and the abdominal cavity was filled with ordinary commercial heparin before closure. The results were gratifying. Lehman and Boys²⁵ (Charlottesville, Va., 1940) have confirmed these experiments and extended them to include animals (rabbits and dogs) in which sepsis was produced by cutting off the tip of the appendix. Forty per cent of these dogs died from peritonitis and 11 per cent had local abscesses. In 30 animals which had healed in six weeks, the adhesions were counted and dissected, and hemostasis was secured. In addition to controls, three types of solution were tried in the abdominal cavity. Saline gave poorest results on re-examination two weeks later. Ascitic fluid was little better than the controls. A definite lessening in the recurrence of adhesions was noted when the Toronto heparin was used. In 1936 Widerström and Wilander² (Stockholm) had shown excellent results with Jorpes' heparin in the prevention of pleural adhesions in rabbits after thoracoparacentesis and injection of iodine.

Heparin is also advocated for Hnas' *exchange transfusion* (blood washing) in uremias,⁵ especially reflex anurias, where the object is to dialyze off the non-protein nitrogen retention products from the blood. The older methods had an anastomosing loop of collodion tubing immersed in warm Ringer-Locke's solution. Later techniques called for the dialysis of successive amounts of blood, heparinized in vitro, with subsequent reinjection.

With regard to its use in ordinary transfusions,⁵ heparin ranks somewhere between the citrate and whole blood methods.²⁶ It is more costly, and there is some danger of incomplete inhibition of coagulation. Heparin is not a good preservative for stored blood. There is little, if any, effect on the recipient's blood as the heparin is quickly diluted down and soon inactivated by heparinase. It is said to be safe even when there has been severe hemorrhage (bleeding ulcers, for instance).

Another method is to heparinize the donor (Hedenius, 1936) with 1 mg. of heparin (100 units) per kilogram intravenously.⁵ No untoward reactions are experienced by donor or recipient. The donor must not risk injury for a couple of hours. It is easier to get sufficient blood without interference due to clotting in the vein. The technique may be simplified. Indeed, in emergencies, the whole procedure may be carried out entirely by means of a few sterile syringes.

As a *blood preservative* for biochemical and other purposes, heparin has advantages and disadvantages. It is anticomplementary. Many of its applications are still in the experimental stage and the scope of its clinical usefulness is especially a problem for the future to decide.

REFERENCES

1. McLean, J.: The Thromboplastic Action of Cephalin, *Am. J. Physiol.* 41: 250, 1916.
2. Howell, W. H.: The Coagulation of Blood, The Harvey Lectures, Series XII: 273, 1917.
Theories of Blood Coagulation, *Physiol. Rev.* 15: 435, 1935.
3. Best, C. H.: Thrombosis and the Action of Heparin, The Harvey Lectures, Series XXVI: 1940. In press.
4. Fischer, A.: Studies on Coagulation of the Blood, *Jap. J. Exper. Med.* 13: 223, 1935.

5. Jorpes, J. E.: Heparin: Its Chemistry, Physiology and Application in Medicine, London, 1939, Oxford Univ. Press.
6. Ferguson, J. H.: Blood: Coagulation, Biophysical Characters, and Formed Elements, *Ann. Rev. Physiol.* 2: 71, 1940; The Role of Blood Clotting Anomalies in the Hemorrhagic Diseases, *J. LAB. & CLIN. MED.* 26: 52, 1940.
7. Wöhlisch, E.: Die Physiologie und Pathologie der Blutgerinnung, *Ergebn. d. Physiol.* 28: 443, 1929.
8. Glazko, A. J., and Ferguson, J. H.: Inhibition of Trypsases by Heparin, *Proc. Soc. Exper. Biol. & Med.* 45: 43, 1940.
9. Glazko, A. J., and Ferguson, J. H.: Kinetics of Thrombin Inactivation as Influenced by Physical Conditions, Trypsin and Serum, *J. Gen. Physiol.* 24: 169, 1940.
10. Ferguson, J. H.: The Action of Heparin, Serum Albumin (Crystalline), and Salmine on Blood-Clotting Mechanisms (in Vitro), *Am. J. Physiol.* 130: 759, 1940.
11. Glazko, A. J., and Ferguson, J. H.: Quantitative Effects of Immediate Antithrombins, *Am. J. Physiol.* In press.
12. Ferguson, J. H.: Heparin and Plasma Albumin in Relation to Thromboplastic Action of Trypsin, Cephalin and Brain Extracts, *Proc. Soc. Exper. Biol. & Med.* 42: 33, 1939.
13. Ferguson, J. H.: Heparin and Natural Antiprothrombin in Relation to Activation and "Assay" of Prothrombin, *Am. J. Physiol.* In press.
14. Ferguson, J. H., and Erickson, B. N.: The Coagulant Action of Crystalline Trypsin, Cephalin and Lung Extracts, *Am. J. Physiol.* 126: 661, 1939.
15. Fiseher, A.: Zum Mechanismus der Hemmung der Blutgerinnung, *Biochem. Ztschr.* 240: 364, 1931.
16. Chargaff, E.: Studies on the Chemistry of Blood Coagulation. VII. Protamines and Blood Clotting, *J. Biol. Chem.* 125: 671, 1938.
17. Mason, M. F.: Heparin: A Review of Its History, Chemistry, Physiology and Clinical Applications, *Surgery* 5: 451, 618, 1939.
18. Murray, D. W. G., Jaques, L. B., Perrett, T. S., and Best, C. H.: Heparin and the Thrombosis of Veins Following Injury, *Surgery* 2: 163, 1937; Murray, D. W. G.: Heparin in Thrombosis and Embolism (Hunterian Lecture), *Brit. J. Surg.* 27: 567, 1940; Murray, D. W. G.: Heparin in Surgical Treatment of Blood Vessels, *Arch. Surg.* 40: 307, 1940.
19. Jaques, L. B., Charles, A. F., and Best, C. H.: The Administration of Heparin, *Acta med. Scandinav. supp.* 90, p. 190, 1938.
20. McLure, R. D., and Lam, C. R.: Experiences in Heparin Administration, *J. A. M. A.* 114: 2085, 1940.
21. Holmin, N., and Ploman, K. G.: Thrombosis of Central Vein of the Retina Treated With Heparin, *Lancet* 1: 664, 1938.
22. Kelson, S. R., and White, P. E.: A New Method of Treatment of Subacute Bacterial Endocarditis Using Sulfapyridine and Heparin in Combination. A Preliminary Report, *J. A. M. A.* 113: 1700, 1939.
23. Knisely, M. H., Thomas, W. K. S., and Eliot, T. S.: *In vivo* Studies of the Progressive Pathological Changes in Experimental Malaria of Monkeys: Microscopic Observations of the Changes in the Blood and Circulation in the Small Vessels of Internal Organs in Living Animals (Motion Pictures), *South. M. J.* 34: 119, 1941.
24. Miki, H., and Satani, H.: Heparin zur Verhütung peritonealer Verklebung, *Mitt. a. d. med. Akad. zu Kyoto* 14: 337, 1935.
25. Lehman, E. P., and Boys, F.: The Prevention of Peritoneal Adhesions With Heparin: An Experimental Study, *Ann. Surg.* 111: 427, 1940.
26. Riddell, V. H.: Blood Transfusion, London, 1939, Oxford Univ. Press, pp. 187-191.

CLINICAL AND EXPERIMENTAL

EXPERIMENTAL STUDIES IN CARDIOVASCULAR PATHOLOGY*

II. PATHOLOGIC LESIONS IN ORGANS OF CATS, GUINEA PIGS, AND FROGS PRODUCED BY DIGITALIS POISONING

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RECENT investigations of Weese and Dieckhoff¹ on the one hand, and of Büchner² as well as Bauer and Fromherz³ on the other hand, have again raised the controversial question as to whether the toxic effects exerted by digitalis glycosides upon the functional activity of the heart muscle are caused by the storage and cumulation of these substances in these elements, as contended by Hatcher,⁴ or whether they are the result of myocardial lesions directly or indirectly produced by these glycosides and thus represent, after repeated administration of digitalis preparations, manifestations elicited by the superimposition of successive anatomic myocardial lesions produced by the individual doses of the digitalis glycosides administered. The conception of an additive anatomic effect as the cause of toxic symptoms from repeated digitalization is based upon the demonstration of hemorrhagic, degenerative, and necrotic myocardial lesions in the hearts of dogs, cats, rabbits, and frogs following the introduction of sublethal and lethal doses of various digitalis preparations (Lewitzki,⁵ Büchner,² Bauer and Reindel,⁶ Hu, Lien, and Li,⁷ Korth and Spang,⁸ and Lindner)⁹.

It is evident that a clarification of the action mechanism of digitalis glycosides, particularly in "cumulative" respects, is of utmost clinical importance since these drugs are used frequently over prolonged periods, until toxic symptoms become apparent. The clinical and pharmacologic observations cannot be correlated satisfactorily with the pathologic data available on this subject because the pathologic studies have been restricted to the heart. This organ was found to be without any myocardial lesions in some animals dying from subacute digitalis poisoning, and exhibited severe anatomic changes in other animals. An experimental reinvestigation of the pharmaco-pathologic interrelations of digitalis poisoning appeared to be indicated wherein special emphasis was to be placed upon a detailed pathologic study of all internal organs.

EXPERIMENTAL.

A purified digitalis preparation, representing the total activity of the leaf, was used in the experiments. Guinea pigs and cats received the drug by intramuscular injection, while frogs were subjected to administrations into the lymph sac. The study consisted of three sets of experiments. Eight guinea

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pigs were injected with a single lethal dose of the digitalis preparation. Sixteen cats received intramuscular injections of one-fifth (10 cats) to one-tenth (6 cats) of the single lethal dose daily until death. Thirty-three frogs were injected daily with the solution of the purified digitalis preparation. The daily amount administered to the frogs was equivalent to one-tenth of the 50 per cent mortality dose for the first twenty-eight days of the experiment and was then increased to one-fifth of the dose for the following five days, after which the treatment was discontinued for the surviving 22 animals.

Series I. Single Lethal Dose.—Two of the 8 guinea pigs died within one to two days after the injection of the lethal dose of the digitalis preparation; 4 died after three to four days; 2 after six to ten days. Post-mortem findings: The lungs were congested and edematous, containing scattered hemorrhagic areas. The left ventricle was firmly contracted, appearing occasionally paler than the excessively dilated right ventricle. The subepicardial vessels were markedly hyperemic. The suprarenals were enlarged and congested and contained medullary hemorrhages in 3 animals. The internal organs, especially the liver, mesentery, intestine, and meninges, exhibited congested blood vessels.

Histology.—Heart: No myocardial abnormalities were found in the hearts of the 2 guinea pigs which died within the first forty-eight hours. One of them, however, showed several small subendocardial hemorrhages. The hearts of 3 of the 4 animals dying within forty-eight to ninety-six hours were free from degenerative myocardial lesions. Small leucocytic foci were present in the myocardium of the fourth animal, in which also a few scattered hyaline degenerated muscle cells were observed. Extensive and multiple areas of hyaline degeneration and necrosis often associated with leucocytic infiltrations were found in the hearts of the 2 guinea pigs which died six to ten days after the injection. Several necrotic foci showed calcium incrustations. There were subepicardial hemorrhages in both animals and subendocardial hemorrhages in one.

Lung: Venous congestion, pulmonary edema, and intra-alveolar hemorrhages were present in all animals.

Liver: Congestion of the hepatic vessels was especially marked in the peripheral zones, where hemorrhages were found occasionally. Multiple, large hyaline necroses existed in the liver of the guinea pig which survived for ten days.

Suprarenal Glands: In addition to the marked congestion and edema present in every case, there existed a hemorrhagic destruction of the medulla in the suprarenal glands of 3 guinea pigs.

Testes: A moderate degeneration of the spermatogenic epithelium was found in 3 animals.

Brain: Only the brain of the guinea pig which died on the sixth day was studied. The meningeal and cerebral vessels were congested markedly, and the cerebral parenchyma was edematous. Small hemorrhages were observed in the region of the floor of the fourth ventricle, where also vacuolated and disintegrating swollen ganglion cells were noted scattered in several centers.

The other organs were normal with the exception of varying degrees of hyperemia.

Series II. Repeated Sublethal Doses.—Symptomatic Observations: The cats reacted after the first injection of the digitalis glycosides with increased salivation, nausea, emesis, and signs of general discomfort, rarely assuming the degree of retching movements or marked general distress. These symptoms disappeared in general within the first twenty-four hours, when loss of appetite, drowsiness, and apathy set in and continued in most animals until death occurred. New attacks of vomiting, accompanied sometimes by diarrhea, were seen, however, in a few cats following repeated injections. Spastic convulsions did not occur. It was noted that the heart action became progressively rapid and irregular during the course of the experiment, so that a "racing heart" and signs of heart block existed in most animals for some time before death. Of the 6 cats which received daily one-tenth of the single lethal dose, one died after four injections, one after seven injections, and 4 after ten to twelve injections. Of the 10 cats composing the group receiving daily one-fifth of the single lethal dose, 6 died after three to five injections, 3 survived six to eight injections, and one died after fifteen injections.

Pathologic Observations.—The post-mortem examinations showed that the hearts had a hard and firmly contracted left ventricle, while the right side of the heart, and especially the right auricle, were dilated. The subepicardial veins were engorged, whereas the coronary epicardial arteries were collapsed and appeared as white strands between the swollen and projecting accompanying veins. Small dark red hemorrhagic areas in the subepicardial tissue were found in one heart near the circular sulcus. A serohemorrhagic pericardial fluid was observed in 3 cats. The lungs were congested and sometimes contained small scattered hemorrhagic areas. The other internal organs were without any appreciable pathologic changes. The meningeal vessels were hyperemic.

Histology.—Heart: Marked pathologic changes were present in the left ventricular wall, the interventricular septum, and especially in the papillary muscle of the left ventricle of the hearts of the 5 cats which died three to five days after the start of the experiment. The lesions were located mainly in the subendocardial region and near the apex. The right ventricular wall as well as the walls of the auricles exhibited only occasionally myocardial changes. There were areas composed of swollen and hydropic muscle cells revealing a rarefaction of the sarcous content (Fig. 1). In other parts there was a more or less marked interstitial edema, proliferation of histiocytes and of fibroblasts present replacing degenerated muscle cells. Multiple foci contained fragmented, partly hyalinized or rarefied muscle cells showing not infrequently a tortuous shape or having cross bands of sarcous granular densifications (contraction bands) (Fig. 2). Scattered calcifications were found in smaller lesions of coagulation necrosis. Hemorrhagic lesions containing muscular fragments in a state of granular or lytic disintegration and infiltrated with leucocytes and lymphocytes were repeatedly seen in the subendocardial zone (Fig. 3). There was an edematous swelling of several medium-sized arterioles (Fig. 4). The subepicardial and myocardial coronary arteries were firmly contracted, while the veins were dilated and engorged. Capillaries located within the degenerated and necrotic areas sometimes showed swollen and thickened walls lined with a proliferated endothelium.

In contrast to these very extensive and severe myocardial lesions present in these 5 cats, the tissues exhibited only minor changes in 2 additional cats dying after three to five injections. Similarly, there were only marked hemorrhagic, degenerative, and necrotic tissue reactions found in the hearts of 2 of the



Fig. 1.—Hydropic and swollen myocardial cells with rarefaction of the sarcous matter.

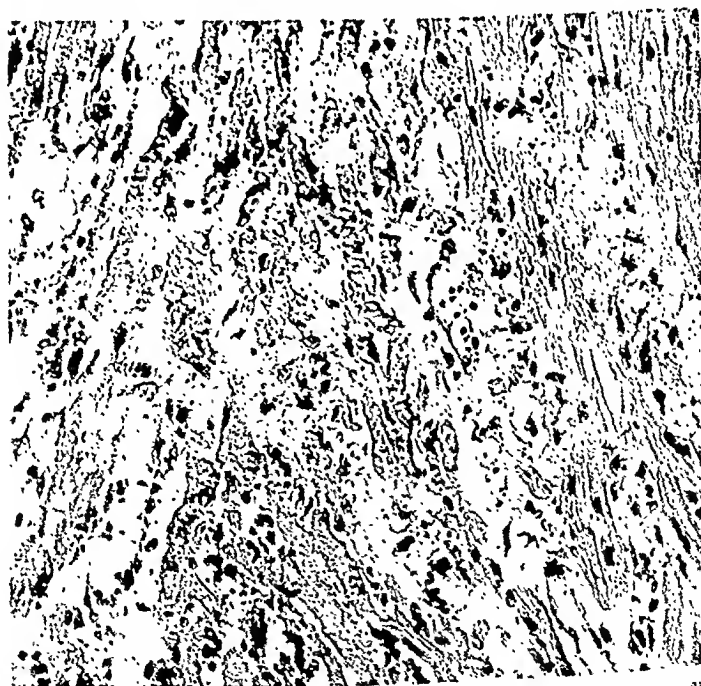


Fig. 2.—Fragmentation and hyaline and vacuolar degeneration of the myocardial cells with interstitial edema and leucocytic infiltration.

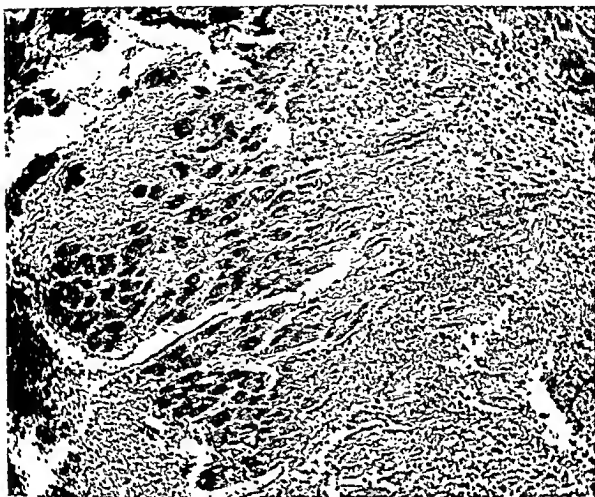


Fig. 3.—Subendocardial necrosis and hemorrhage with marked leucocytic infiltration into the edematous tissue containing partially lysed muscle cells.



Fig. 4.—Myocardial arteriole with swollen and edematous wall.

9 cats which died after six to fifteen injections, while the hearts of the other 7 animals exhibited only scanty and mild degenerative lesions. Numerous small fibroblastic scars, in addition to extensive regressive and inflammatory myocardial lesions, were observed in the heart of the cat which died after fifteen injections.

(Glycogen stains of the heart sections made with the Best's carmine method were negative in four instances and showed mere traces in a fifth case.

Lung: The veins were markedly engorged, the arteries were collapsed, and the pulmonary parenchyma showed intra-alveolar edema and scattered hemorrhages.

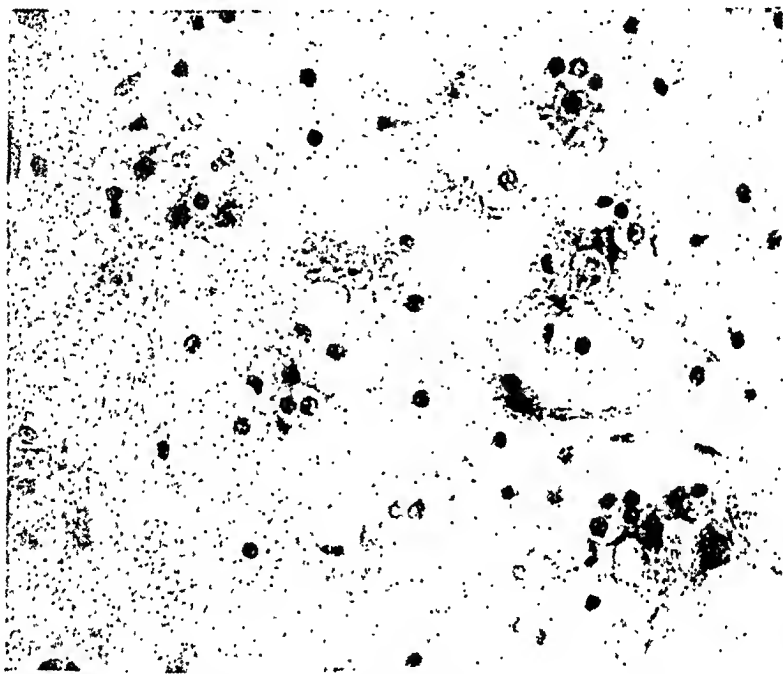


Fig. 5.—Degenerated ganglion cells and neuronophagia in a center located at the floor of the fourth ventricle.

Liver: In addition to marked, especially pericentral congestion, there existed interstitial edema and hemorrhages accompanied by hepatic atrophy in several cases. Glycogen stains were negative.

The other internal organs (kidney, spleen, suprarenals, pancreas, lymph nodes, intestine, stomach, ovary, bone marrow, uterus, etc.) showed varying degrees of congestion.

Brain: There were degenerative glial and ganglionic cellular changes present in the majority of cats. These lesions were remarkable, particularly in some of the cats which died with minor myocardial degenerative manifestations. The lesions consisted of vacuolization and disintegration of individual ganglion cells located in the centers of the basal region and in the brain stem (Fig. 5). In two instances smaller glial cell infiltrations were found in the cerebellum (Fig. 6), while markedly engorged capillaries with focal degeneration of the nervous substance were seen occasionally (Fig. 7).

Series III. Prolonged Repeated Injections.—Five frogs lived for four to six days, 4 for ten to twenty days, 2 for twenty-nine to thirty-one days, 16 for forty to forty-three days, and 6 for seventy to seventy-five days. All frogs which survived for more than forty-two days were killed. The hearts of the majority of the frogs which died spontaneously showed a firmly contracted grayish red



Fig. 6.—Glial focus in the cerebellum.

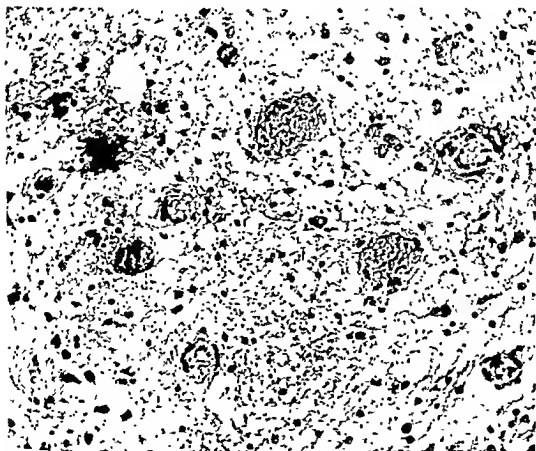


Fig. 7.—Congested capillaries surrounding a focus of degenerated nervous substance.

to pale yellowish-gray ventricle, which seemed to be attached like a small nodule to the markedly distended and engorged auricle. The lungs of these animals were often congested. The stomach was distended and contained a thick jellylike grayish white matter. The organs of the animals which were sacrificed after the arrest of the treatment exhibited normal organs.



Fig. 8.—Contraction bands in the heart of a frog.

Histology.—Heart: The most characteristic and frequent, while by no means constant abnormality found in the hearts of the frogs which died during treatment was a marked swelling and hydropic condition of the muscle cells of the ventricle. Contraction bands consisting of hyaline, granular material, which was indistinctly demarcated from the adjacent hydropic sarcolemmal content of the cells, were running crosswise to the longitudinal axis of muscle bundles (Fig. 8). Because of the firm contraction of the ventricle very little blood was present in between the nonvascularized muscular trabeculae composing the wall of the frog heart. There were no degenerative lesions.

The liver and kidney were congested and often edematous.

The internal organs of the frogs killed after cessation of the treatment were normal.

COMMENT

The pathologic observations made regarding the presence and type of myocardial lesions found in animals dying from digitalis poisoning confirm in general those previously recorded. They substantiate, moreover, the statements of Bauer and Reindell,⁶ and Lindner,² that such anatomic myocardial changes are not present in all animals (in 80 per cent, according to Bauer and Reindell) subjected to lethal and sublethal doses of digitalis glycosides. It is obvious, therefore, that myocardial pathology cannot represent in all instances the cause of symptomatic toxic manifestations observed during life and its lethal sequelae, as contended by Büchner,² and Bauer and Fromherz.³ In addition to these

cases in which no appreciable myocardial lesions exist, there are others in which the anatomic changes in the heart after toxic doses of digitalis glycosides are so mild that they cannot be considered as a major etiologic factor in bringing about the lethal results observed. While these considerations seem to discredit the anatomic conceptions of Büchner, and Bauer and Fromherz, and support apparently the functional-cumulative theory of Hatcher,⁴ there remains the possibility that primary or secondary extracardiac organic anatomic lesions, such as described in this presentation, may be involved in or contribute to the causation of the toxic symptomatic reactions, particularly those affecting the heart and the nervous system, and of the fatal end results.

There exists a certain amount of functional and anatomic evidence indicating that such a causative mechanism may play a part in the appearance of the toxic and fatal manifestations associated with digitalis poisoning in animals. It is a well-recognized fact that an overdigitalization leads to an excessively prolonged and accentuated systolic contraction and an incomplete and shortened diastolic dilation of the ventricles. This functional state of the myocardium results in a more or less serious circulatory impairment characterized by a defective filling of the arterial system and a retention of the blood in the venous system. Such a circulatory state produces a relative stagnant hypoxemia in the extracardiac organs followed, when prolonged, by the development of degenerative changes in those organs, such as the brain, which are particularly sensitive to deficiencies in their oxygen supply. This hypoxemic condition is accentuated during attacks of vomiting and convulsions, and during the state of the racing heart, which in turn aggravate the ischemic anoxemia of the myocardium resulting from the compression of the myocardial arterioles by the bulges of the contracted muscle bundles (Anrep,¹⁰ Fock¹¹) and interfering with the functional efficacy of the myocardium. Thus, a vicious circle is established between disturbances of the functional activity of the heart with its resulting circulatory effects and the functional and anatomic changes elicited by these conditions in extracardiac organs. The site (suprarenal, brain) and character of these lesions (hemorrhages, degeneration of ganglion cells in the region of vitally important centers) as well as their potentially progressive nature suggest that they may be the cause not only of prolonged functional cardiac disturbances, but also of the occurrence of the delayed deaths observed in animals without appreciable or severe myocardial pathology.

In view of the complete absence of corresponding observations in connection with the toxic reactions associated with a therapeutic overdigitalization in man, it remains uncertain whether a similar vicious circle of functional and anatomic reactions is produced and may contribute to the untoward effects noted sometimes in patients subjected to this type of treatment.

CONCLUSIONS

The introduction of single lethal doses of digitalis glycosides into guinea pigs, as well as of repeated sublethal doses of the drug into cats, results in the production of degenerative and necrotic myocardial and extracardiac (cerebral, suprarenal, hepatic, renal) organic lesions in the majority of the animals treated.

Some of the animals, however, dying from the toxic effects of the digitalis glycosides show at death no appreciable, or only minor cardiac, changes, while the extracardiac, and particularly cerebral, lesions are well developed.

The anatomic evidence obtained indicates that subacute and delayed death from digitalis poisoning may be the result of anatomic, myocardial lesions of severe type or, in the absence of these, of extracardiac cerebral or suprarenal lesions.

REFERENCES

1. Weese, H., and Dieckhoff, J.: Zur Kumulation der Digitalisglykoside, Arch. f. exper. Path. u. Pharmacol. 176: 274, 1934.
2. Büchner, F.: Herzmuskelnekrosen durch hohe Dosen von Digitalisglykosiden, Arch. f. exper. Path. u. Pharmacol. 176: 59, 1934; Herzmuskelnekrosen durch Digitalisglykoside, Klin. Wchnschr. 12: 1237, 1933.
3. Bauer, H., and Fronherz, K.: Über die Kumulierung der Digitalis-Glykoside, Klin. Wchnschr. 12: 973, 1933.
4. Hatcher, R. A.: The Persistence of Action of the Digitalins, Arch. Int. Med. 10: 263, 1912.
5. Lewitzki: Über pathologisch-histologische Veränderungen des Herzens bei Digitalisvergiftung, Inaug. Diss., Petersburg, 1904. Abstr. zentrallbl. f. Path. 16: 532, 1905.
6. Bauer, H., and Reindell, H.: Zur Kenntnis der Ursachen der Kumulierungserscheinungen der Digitalisglykoside, Arch. f. exper. Path. u. Pharmacol. 191: 311, 1939.
7. Hu, C. H., Lieu, V. T., and Li, R. C.: Anatomical Changes in the Hearts of Dogs and Frogs Poisoned by Glucosides of the Digitalis Group, Chinese M. J. (suppl.) p. 31, 1936.
8. Korth, C., and Spang, K.: Die Wirkung des Digitoxins auf Elektrokardiogramm und Herzmuskel der Katze, Arch. f. exper. path. u. Pharmacol. 184: 349, 1937.
9. Lindner, W.: Untersuchungen zur Kumulation der Digitaliskörper, Arch. f. exper. Path. u. Pharmacol. 192: 155, 1939.
10. Anrep, G. V.: Der Einfluss der Herzkontraktion auf den Blutstrom in den Coronararterien, Klin. Wchnschr. 12: 1353, 1933.
11. Fock, H. I.: Über Cyanose des Herzens, Inaug. Diss., Berlin, 1932.

FURTHER STUDIES ON THE BEHAVIOR OF GLUCONIC ACID AND AMMONIUM GLUCONATE IN ANIMALS AND MAN*

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THE present report deals with a study of certain aspects of the pharmacology of gluconic acid, in particular its effect on the pH of the urine, its excretion in the urine, and its toxicity by oral and intravenous injection in acute and chronic experiments.

Mechanism of Increased Urinary Acidity After Gluconic Acid.—In a previous study¹ we found that large oral doses of gluconic acid† administered to human subjects sometimes increased the acidity of the urine, the pH falling from 0.1 to 1.0 unit. Such a decline in the pH need not necessarily be due to excreted gluconic acid, since the administration of an organic acid which shifts the acid-base equilibrium of the blood toward the acid side may result in increased

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†Throughout these studies the preparation used was glucono-delta-lactone of Charles Pfizer & Co., Inc.

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excretion of acid phosphates, and this factor may be responsible for the shift in the pH of the urine. We pointed out in the previous study that the fate of gluconic acid is not established, some studies indicating a high degree of excretion and others the possibility that it is chiefly destroyed. We, therefore, performed experiments to ascertain the role of excreted gluconic acid in producing the changes in the pH of the urine.

In the first group of experiments gluconic acid was added to urine of 6 normal subjects and of one with a urinary infection, in concentrations varying from 0.1 to 5 per cent gluconic acid. Forty-three pH determinations were made. The results showed a fall in the pH of from 0.1 to 1.2 units. All pH determinations were made with the Leeds and Northrup potentiometer using the glass electrode. In general, the higher the concentration of gluconic acid, the greater the decrease in the pH of the urine. Marked variations were in evidence, however, among different specimens of urine. For example, in each of two urines, in which the pH decreased by 0.6, the change was brought about by a 0.9 per cent solution of gluconic acid in the one and by a 5 per cent solution in the other. A 1 per cent solution of gluconic acid lowered the pH by as little as 0.1 in one urine, while the same change of pH was produced by a much more dilute solution of gluconic acid, 0.2 per cent, in another specimen. The original pH is not the sole factor that determines these variations, since two urines having the same pH at the start may show quite different changes with the same concentrations of gluconic acid. For example, in the case of two urines each of which had a pH of 5.9, one showed a decrease of 0.9 and the other a decrease of 0.6 with a gluconic acid concentration of 2.5 per cent; similarly, when the concentration of gluconic acid was raised to 5 per cent in these two, the pH decreased by 1.2 in one and by 0.9 in the other. These differences, as might be expected, are due to the different buffering powers of various urines.

These experiments showed that the gluconic acid content of urine after large oral doses in man should be 0.3 per cent or higher in order to produce an appreciable fall in the pH of the urine, such as, for example, 0.1 to 0.3 unit.

Gluconic acid is much less effective than mandelic acid in lowering the pH. In one specimen of urine a 5 per cent solution of gluconic acid produced a smaller effect (fall of pH by 1.2) than a 1 per cent solution of mandelic acid (fall of pH by 2.5).

GLUCONIC ACID EXCRETION IN URINE AFTER ORAL ADMINISTRATION

Experiments were then performed to determine the gluconic acid content of urine in human subjects after the oral administration of large doses.

An attempt was first made to determine the gluconic acid by the preparation of its phenylhydrazide, according to the technique described by Fischer and Passmore,² and modified by Kiliani³ and Van Marle.⁴

In one experiment in which 200 mg. of gluconic acid were added to 25 c.c. of distilled water, a yield of 98 per cent of the theoretical quantity of the phenylhydrazide was obtained. It gave the melting point of gluconic acid phenylhydrazide, namely, 200° C. Each of two specimens of urine was divided into four 25 c.c. portions, and gluconic acid was added in amounts varying from 200

to 400 mg. The yield varied from 10 to 69 per cent of the added gluconic acid for the 8 samples, and from 18 to 69 per cent for the samples of the same specimen.

An attempt was made to recover gluconic acid phenylhydrazide from the urine collected three hours after the oral administration of 5 to 20 Gm. of gluconic acid in 5 persons. None was recovered in 3. In 2 others the recoveries were 10 and 160 mg., respectively. The crystals were impure. These results showed that the phenylhydrazide method applied to urine yields hydrazides other than that of gluconic acid and is not a satisfactory method for determining the excretion of gluconic acid in urine. As far as the results go, however, they indicate that very little of a large oral dose of gluconic acid is excreted in the urine in man.

In another series of experiments an attempt was made to determine the urinary excretion of gluconic acid polarimetrically by means of the alkaline molybdate complex. De Carli⁵ showed that the specific rotation of gluconic acid is very low. However, Lütz and Jirgensons,⁶ and Schmidt and Weber-Molster⁷ showed that the specific rotation of various aldonic acids can be greatly increased by forming complexes with sodium or ammonium molybdate. Bennet-Clark⁸ employed acid solutions of ammonium molybdate for determining gluconic acid in plant extracts. The acid solution, however, is not applicable to urine because of other substances normally present which color the molybdate, and Lütz and Jirgensons⁶ showed that the greatest rotation in the case of gluconic acid is obtained in alkaline solution.

The urine was first cleared by centrifuging twice at 4,000 r.p.m. It was decanted into a fine filter and allowed to filter slowly in the refrigerator. Charcoal or other similar agents for clearing urine are undesirable in the case of gluconic acid.⁹ Eight cubic centimeters of the clarified urine were placed in a 50 c.c. Erlenmeyer flask. To this 5 c.c. of a saturated aqueous solution of ammonium molybdate were added, and 0.5 c.c. of 24 per cent sodium hydroxide. This was made up to 25 c.c. with distilled water. It was well mixed and allowed to stand in the dark for about one hour, although the indications are that accurate readings can be made in from thirty minutes to twenty-four hours. A determination

TABLE I

EFFECT OF THE CONCENTRATION OF GLUCONIC ACID ON THE DEGREE OF OPTICAL ROTATION IN URINE

CONCENTRATION OF GLUCONIC ACID LACTONE (%)	ANGLE OF ROTATION
2.0	3.49
1.5	2.55
1.0	1.66
0.9	1.47
0.8	1.30
0.7	1.15
0.6	0.97
0.5	0.80
0.4	0.64
0.3	0.46
0.2	0.32
0.1	0.17
Normal urine	-0.02
Distilled water	0.00

of the relationship between the concentration of gluconic acid lactone in urine and the angle of rotation showed that with concentrations between 0.1 and 2.0 per cent this relationship is a straight line function (Table I).

The polarimetric method of determining gluconic acid in urine was applied to four experiments in a dog and to thirty experiments in six persons. Illustrative data are presented in Table II. A normal female dog was placed in a metabolism cage and kept on a normal stock diet with unlimited water. The bladder was emptied before the drug was administered. Urine specimens were collected from the bladder by means of a catheter at intervals of from one-half to seven and a half hours after the dose. To insure a satisfactory volume of urine, 100 c.c. of tap water were placed in the stomach after the doses of the drug in three of the four experiments. Each experiment was done on a different day. The following doses were used: 0.5 Gm. of gluconic acid per kilogram intravenously in two experiments, 1 Gm. per kilogram by stomach tube in the third, and 1.5 Gm. per kilogram by stomach tube in the fourth. The pH of the urine specimens collected from one-half to five hours after the dose was usually less than 5.5, and ranged from 4.8 to 6.0. The results showed that the dog excretes some gluconic acid in the urine, more after intravenous injections than after oral administration. In the case of the intravenous injections, most of the excretion took place in the first half hour. The total amount excreted in the urine in either case was small, namely, 1.1 and 2.4 per cent of

TABLE II

URINARY EXCRETION OF GLUCONIC ACID DETERMINED POLARIMETRICALLY

DATE	DOSE* (GM./ KG.)	MODE OF ADMINIS- TRATION	INTERVAL† (HR.)	pH OF URINE	CONCEN- TRATION OF GLUCONIC ACID IN URINE SAMPLE (%)	GLUCONIC ACID RECOVERED IN EACH SAMPLE (% OF DOSE)	TOTAL GLUCONIC ACID RECOVERED AFTER EACH DOSE (% OF DOSE)
<i>In the dog</i>							
8/23	1	Stomach tube	4.5 7.5	6.0 —	0.11 0.01	1.2 0.075	2.4
8/31	0	—	—	4.5	0	0	5.4
	0.5	Vein	0.5	4.8	4.4	3.1	
	—	—	1	5.8	0.6	1.4	
	—	—	1.5	4.9	1.3	0.9	
	—	—	2	5.5	0.22	0	
<i>In man</i>							
8/21	(Gm. total) 10	Oral	3.5 7.0 24.0	5.7 — —	0.25 0.26 0	3.5 4.2 0	7.7
8/22	10	Oral	3.5 7.0 24.0	5.9 — —	0.33 0.05 0	10.9 1.8 0	12.7
8/24	10	Oral	3.5 7.0 24.0	5.9 — —	0.58 0.24 0	6.4 8.6 0	15.0

*Both oral and intravenous doses were given as a 10 per cent solution of the delta lactone of gluconic acid.

†These time intervals represent the period from the administration of the drug to the collection of the sample of urine.

preciable sustained injury is equivalent to an intravenous injection of 30 Gm. for the average man on the basis of body weight. It is clear, therefore, that the toxicity of ammonium gluconate or gluconic acid by intravenous injection is very low.

Effect of Gluconic Acid on the Urine of Human Subjects.—The urines of 5 normal persons were examined for protein, casts, blood cells, pus cells, and sugar. The subjects were then given daily doses of gluconic acid of 5 to 10 Gm. each for periods varying from three to six days. At the end of this period the urine was re-examined in the same way. The results show that gluconic acid produces no signs of renal injury, judged in this way.

Effect of Gluconic Acid on the pH of the Urine.—In the previous study¹ the results of experiments were presented showing that the delta lactone of gluconic acid given in large doses reduced to low levels the pH of the urine in normal human subjects. The dose was 15 Gm. of gluconic acid given orally on an empty stomach. The bladder was emptied at the time the dose was taken, and the urine secreted during the first four-hour period after the dose was examined for the pH. Inasmuch as the larger doses had a fairly marked tendency to cause cramps and diarrhea, similar experiments were now made with 5 Gm. doses in order to see whether changes in the pH of the urine could be produced without the gastrointestinal symptoms. These experiments were carried out in 10 persons, 5 with and 5 without a urinary infection. Control specimens of urine were examined for the pH under the same conditions as those after the dose of the drug. In the different patients the average pH for the controls was based upon the results in from five to sixteen specimens, and after the gluconic acid the average pH was based upon the results in from five to thirty-seven specimens. The results with the 5 Gm. doses were equivocal. The doses were then increased to from 10 to 50 Gm. given at one time. Marked variations in the changes of the average pH of the urine after the drug were observed. In the different cases they were as follows: +0.9, -0.2, +0.4, +0.1, -0.3, -0.5, 0.0, -0.7, -0.1, and -0.9. Urinary infection was present in the first five of these cases. It appears, therefore, that normal and infected urines do not behave alike. In normal urine the pH usually declined. In infected urine a decline rarely occurred. In fact, the usual response was a rise of the pH. A similar resistance of infected urine to acidification by intravenous injections of gluconic acid in human beings was reported by Bodon.¹⁰

Effect of Ammonium Gluconate.—In a group of 8 persons the effect of ammonium gluconate on the pH of the urine was tested. There were 2 with infected urine and 6 with normal urine. The experiments were carried out in a manner similar to those in which the delta lactone of gluconic acid was used. The doses were from 5 to 30 Gm., given in the form of a powder, dissolved in 8 ounces of water, with the urinary bladder empty, four hours before the specimen of urine was collected. The results showed that ammonium gluconate in such doses as these also tends to reduce the pH of normal urine, and in the two subjects with infected urine, the pH did not fall, but rose. Four of the 8 patients developed nausea, cramps, and diarrhea. These disagreeable effects of ammonium gluconate, therefore, are similar to those encountered during the use of gluconic acid.

Illustrative data of the effect of gluconic acid and ammonium gluconate on the pH of the urine in man are presented in Table III.

TABLE III

EFFECT OF INGESTED GLUCONIC ACID AND AMMONIUM GLUCONATE ON THE pH OF URINE IN MAN

NO.	SUBJECT	TOTAL DAILY DOSE (GM.)			NO. SPECIMENS IN CONTROLS	NO. SPECIMENS AFTER DRUG	PH DETERMINA- TIONS		AVERAGE PH IN CONTROLS	AVERAGE PH AFTER DRUG	AVERAGE CHANGE IN PH DURING TREATMENT
							RANGE IN CONTROLS	RANGE AFTER DRUG			
Gluconic Acid											
1	Fro*	5(5)†	10(11)	20(6)	5	22	5.7-5.2	7.5-5.4	5.5	6.4	+0.9
2	Edw*	5(3)	10(28)	20(5)	12	36	6.9-5.7	7.3-5.2	6.4	6.8	+0.4
3	Mod	5(4)	10(1)	15(5)	16	10	7.0-5.1	5.9-4.9	5.7	5.2	-0.5
4	Tra	5(5)	10(5)	15(5)	10	15	6.2-4.9	6.2-4.8	5.7	4.8	-0.9
Ammonium Gluconate											
1	Gre*	10(2)	20(10)	30(4)	8	16	7.5-7.1	8.1-7.1	7.1	7.4	+0.3
2	Rei*	5(6)	10(3)	20(7) 30(4)	10	19	6.2-5.6	6.6-5.0	5.9	6.1	+0.2
3	Jon	5(6)	10(3)	20(3)	17	12	7.2-5.4	6.1-5.0	6.0	5.5	-0.5
4	Mac	5(6)	10(3)	—	7	9	6.9-5.2	6.2-4.9	6.4	5.5	-0.9

*Subjects with urinary infection; others without urinary infection.

†These show number of days drug was taken.

SUMMARY AND CONCLUSIONS

1. A method has been developed for the determination of gluconic acid in urine polarimetrically by means of the ammonium molybdate complex of gluconic acid.
2. It has been determined that the degree of optical rotation produced by the complex of gluconic acid bears a straight line relationship to the concentration of gluconic acid.
3. After large oral doses of gluconic acid the urine, when treated with ammonium molybdate, develops optical rotation. We have assumed it to represent excreted gluconic acid.
4. After 10 to 30 Gm. doses of gluconic acid given orally, human subjects were found to excrete an amount varying from 7.7 to 15.0 per cent of the dose in the succeeding twenty-four hours, the major part of the excretion taking place within the first few hours.
5. After doses of 10 to 30 Gm. of gluconic acid the concentration in the urine is extremely variable, ranging from 0.03 to 2.24 per cent, usually, however, less than 0.5 per cent.
6. In vitro experiments in which gluconic acid was added to the urine showed that the foregoing concentrations could account for the amount of decrease of the pH of the urine which followed the oral doses of gluconic acid. It is inferred, therefore, that the fall of the urinary pH after the administration of gluconic acid is due to the excreted gluconic acid, or some closely allied acid derivative.

1.0 to 1.5 Gm. per kilogram by oral administration, and 5.4 and 6.0 per cent of 0.5 Gm. per kilogram by intravenous injection.

In the human subjects the gluconic acid was given in a single dose of 5, 10, 20, or 30 Gm. orally at 9 A.M., after a constant breakfast at about 8 A.M. The bladder was emptied just before the drug was taken dissolved in 8 ounces of water. No food was taken until after the first sample of urine which was collected, three and a half hours following the drug. The second sample was collected seven hours after the drug, and the third sample represented the pooled urine voided up to twenty-four hours after the drug. Variable amounts of gluconic acid appeared in the urine after its oral administration. None was recovered after a 5 Gm. dose in two experiments. After from 10 to 30 Gm. doses, 0.5 to 17.5 per cent appeared in the urine. The recovery varied greatly in the same individual after a similar dose and similar interval on different days. For example, in one subject who received a 10 Gm. dose on each of five days, the recovery of gluconic acid in the urine three and a half hours after each dose varied as follows: 1.4, 2.2, 2.6, 0.0, 5.7 per cent of the dose. The largest amount usually appeared in the first seven hours after the ingestion. In four experiments in which the total excretion was determined, it varied from 7.7 to 15 per cent of the 10 Gm. dose.

The concentration of gluconic acid in the urine of the dog varied from 0.01 to 4.4 per cent. In human subjects in whom the doses were smaller on the basis of body weight, the concentrations ranged from 0.03 to 2.24 per cent.

The decrease in the pH of the urine after gluconic acid in oral doses of from 5 to 50 Gm. ranged from 0.1 to 1.0. When gluconic acid was added to urine, it was found that concentrations of from 0.1 to 0.9 per cent produced decreases in the pH of the order of 0.1 to 0.6 unit. Since such concentrations of gluconic acid appear in the urine after oral doses, we may conclude that the pH of the urine following the oral administration of gluconic acid could be explained by the excreted gluconic acid as estimated above.

We have observed that control urine does not form an optically active complex with ammonium molybdate in the manner in which these experiments were performed. Furthermore, the urine secreted after the injection of gluconic acid is not optically active until its ammonium molybdate complex has been formed. We have assumed that this optically active compound represents gluconic acid, the amount of which was calculated in terms of the lactone. The possibility that this compound which appears may not be gluconic acid, but some related conversion product which possesses little direct optical activity, but which develops optical rotation as its ammonium molybdate complex in a manner similar to gluconic acid, has not been excluded.

TOXICITY OF GLUCONIC ACID

In the Cat and the Dog.—Each of 5 cats received a daily dose of 1 Gm. of gluconic acid per kilogram as a 10 per cent solution by stomach tube for fourteen days. Daily observations were made for signs of toxicity, and the urine was examined daily for protein, blood, casts, and sugar. The urine was also examined during a control period of five days prior to the administration of the

drug. The animals were weighed at the beginning and again at the end of the experimental period.

The general appearance of the animals remained unchanged. They retained their control weight. Three developed vomiting and diarrhea on several occasions. The urine remained unchanged.

At the end of the two weeks the animals were killed with ether or chloroform, and on gross examination the lungs, heart, liver, kidneys, gastrointestinal tract, bladder, ureter, and spleen were found normal. Sections of the lungs, liver, and kidneys were prepared, stained with hematoxylin and eosin, and examined histologically. In all there were 126 such sections. These also failed to show any significant changes when compared with the tissues of 2 normal cats examined in the same way.

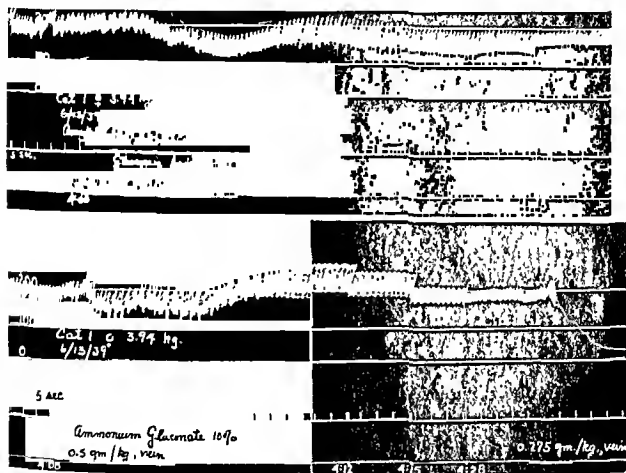


Fig. 1.

A similar series of experiments was carried out on 3 dogs. The results are similar to those obtained in the cats. No signs of ill health developed during the period of two weeks. The urine remained unchanged and at autopsy no significant gross or histologic changes in the tissues (33 sections examined) were found.

Effect of Intravenous Injection in the Cat.—Fig. 1 shows the effect of gluconic acid and ammonium gluconate given by intravenous injection in the cat. A dose of 500 mg. per kilogram produced a temporary fall of the blood pressure, which returned to the normal level within about five minutes. Eight minutes later a similar dose of ammonium gluconate was given. This also produced a temporary fall of the blood pressure, followed by a marked secondary rise. An additional dose of 275 mg. per kilogram given very rapidly about twenty minutes later produced abrupt cessation of the heart. The dose which produced no ap-

preciable sustained injury is equivalent to an intravenous injection of 30 Gm. for the average man on the basis of body weight. It is clear, therefore, that the toxicity of ammonium gluconate or gluconic acid by intravenous injection is very low.

Effect of Gluconic Acid on the Urine of Human Subjects.—The urines of 5 normal persons were examined for protein, casts, blood cells, pus cells, and sugar. The subjects were then given daily doses of gluconic acid of 5 to 10 Gm. each for periods varying from three to six days. At the end of this period the urine was re-examined in the same way. The results show that gluconic acid produces no signs of renal injury, judged in this way.

Effect of Gluconic Acid on the pH of the Urine.—In the previous study¹ the results of experiments were presented showing that the delta lactone of gluconic acid given in large doses reduced to low levels the pH of the urine in normal human subjects. The dose was 15 Gm. of gluconic acid given orally on an empty stomach. The bladder was emptied at the time the dose was taken, and the urine secreted during the first four-hour period after the dose was examined for the pH. Inasmuch as the larger doses had a fairly marked tendency to cause cramps and diarrhea, similar experiments were now made with 5 Gm. doses in order to see whether changes in the pH of the urine could be produced without the gastrointestinal symptoms. These experiments were carried out in 10 persons, 5 with and 5 without a urinary infection. Control specimens of urine were examined for the pH under the same conditions as those after the dose of the drug. In the different patients the average pH for the controls was based upon the results in from five to sixteen specimens, and after the gluconic acid the average pH was based upon the results in from five to thirty-seven specimens. The results with the 5 Gm. doses were equivocal. The doses were then increased to from 10 to 50 Gm. given at one time. Marked variations in the changes of the average pH of the urine after the drug were observed. In the different cases they were as follows: +0.9, -0.2, +0.4, +0.1, -0.3, -0.5, 0.0, -0.7, -0.1, and -0.9. Urinary infection was present in the first five of these cases. It appears, therefore, that normal and infected urines do not behave alike. In normal urine the pH usually declined. In infected urine a decline rarely occurred. In fact, the usual response was a rise of the pH. A similar resistance of infected urine to acidification by intravenous injections of gluconic acid in human beings was reported by Bodon.¹⁰

Effect of Ammonium Gluconate.—In a group of 8 persons the effect of ammonium gluconate on the pH of the urine was tested. There were 2 with infected urine and 6 with normal urine. The experiments were carried out in a manner similar to those in which the delta lactone of gluconic acid was used. The doses were from 5 to 30 Gm., given in the form of a powder, dissolved in 8 ounces of water, with the urinary bladder empty, four hours before the specimen of urine was collected. The results showed that ammonium gluconate in such doses as these also tends to reduce the pH of normal urine, and in the two subjects with infected urine, the pH did not fall, but rose. Four of the 8 patients developed nausea, cramps, and diarrhea. These disagreeable effects of ammonium gluconate, therefore, are similar to those encountered during the use of gluconic acid.

Illustrative data of the effect of gluconic acid and ammonium gluconate on the pH of the urine in man are presented in Table III.

TABLE III

EFFECT OF INGESTED GLUCONIC ACID AND AMMONIUM GLUCONATE ON THE pH OF URINE IN MAN

NO.	SUBJECT	TOTAL DAILY DOSE (GM.)			NO. SPECIMENS IN CONTROLS	NO. SPECIMENS AFTER DRUG	pH DETERMINA- TIONS		AVERAGE pH IN CONTROLS	AVERAGE pH AFTER DRUG	AVERAGE CHANGE IN pH DURING TREATMENT
							RANGE IN CONTROLS	RANGE AFTER DRUG			
Gluconic Acid											
1	Fro*	5(3)†	10(11)	20(6)	5	22	5.7-5.3	7.5-5.4	5.5	6.4	+0.9
2	Edw*	5(3)	10(28)	20(5)	12	36	6.9-5.7	7.3-5.2	6.4	6.8	+0.4
3	Mod	5(4)	10(1)	15(5)	16	10	7.0-5.1	5.9-4.9	5.7	5.2	-0.5
4	Tra	5(5)	10(5)	15(5)	10	15	6.2-4.9	6.2-4.8	5.7	4.8	-0.9
Ammonium Gluconate											
1	Gre*	10(2)	20(10)	30(4)	8	16	7.5-7.1	8.1-7.1	7.1	7.4	+0.3
2	Rei*	5(6)	10(3)	20(7)	10	19	6.2-5.6	6.6-5.0	5.9	6.1	+0.2
				30(4)							
3	Jon	5(6)	10(3)	20(3)	17	12	7.2-5.4	6.1-5.0	6.0	5.5	-0.5
4	Mac	5(6)	10(3)	—	7	9	6.9-5.2	6.2-4.9	6.4	5.3	-0.9

*Subjects with urinary infection; others without urinary infection.

†These show number of days drug was taken.

SUMMARY AND CONCLUSIONS

1. A method has been developed for the determination of gluconic acid in urine polarimetrically by means of the ammonium molybdate complex of gluconic acid.

2. It has been determined that the degree of optical rotation produced by the complex of gluconic acid bears a straight line relationship to the concentration of gluconic acid.

3. After large oral doses of gluconic acid the urine, when treated with ammonium molybdate, develops optical rotation. We have assumed it to represent excreted gluconic acid.

4. After 10 to 30 Gm. doses of gluconic acid given orally, human subjects were found to excrete an amount varying from 7.7 to 15.0 per cent of the dose in the succeeding twenty-four hours, the major part of the excretion taking place within the first few hours.

5. After doses of 10 to 30 Gm. of gluconic acid the concentration in the urine is extremely variable, ranging from 0.03 to 2.24 per cent, usually, however, less than 0.5 per cent.

6. In vitro experiments in which gluconic acid was added to the urine showed that the foregoing concentrations could account for the amount of decrease of the pH of the urine which followed the oral doses of gluconic acid. It is inferred, therefore, that the fall of the urinary pH after the administration of gluconic acid is due to the excreted gluconic acid, or some closely allied acid derivative.

7. Additional experiments with oral doses of gluconic acid confirm the previous results showing that the pH of the urine can be significantly reduced by doses of the order of 10 Gm. or more. Smaller doses produce equivocal results.

8. In a series of similar experiments with ammonium gluconate the pH of the urine was similarly lowered by similar doses.

9. While a significant lowering of the pH occurred almost uniformly in cases of normal urine, the reverse occurred in cases with urinary infections. In the latter the pH rarely fell and usually rose. The reason for this difference requires further study.

10. The acute toxicity of gluconic acid and ammonium gluconate is low. Only temporary fluctuations in the blood pressure are produced by an intravenous injection of a 10 per cent solution of gluconic acid or of ammonium gluconate in a dose of 0.5 Gm. per kilogram, equivalent to a total of about 30 Gm. for a man.

11. The daily oral administration of large doses of gluconic acid to dogs and cats for a period of two weeks failed to produce any gross or histologic tissue changes.

12. The continued administration of large oral doses of gluconic acid to normal human subjects also failed to produce any pathologic renal changes, as evidenced by the absence of blood, protein, casts, and sugar in the urine.

13. Gluconic acid and ammonium gluconate appear to be equally effective in lowering the pH of the urine in man in the absence of urinary infection. To produce this effect, single doses of about 10 Gm. or more are necessary, and these cause gastrointestinal disturbances in about one-half of the cases. In individuals with infected urine the effect of these compounds on the pH of the urine is much less certain, more often causing a diminished acidity.

REFERENCES

1. Gold, H., and Civin, H.: Gluconic Acid as a Urinary Acidifying Agent in Man, *J. Lab. & Clin. Med.* 24: 1139, 1939.
2. Fischer, E., and Passmore, F.: Ueber die Bildung der Phenylhydrazide, *Ber. d. deutsch. chem. Gesellsch.* 22: 2728, 1889.
3. Kiliani, H.: New Observations in the Chemistry of the Sugars, *Chem. Abstr.* 20: 2957, 1926.
4. Van Marle, T. W. J.: Some Relations Between the Configuration and the Optical Rotatory Power of Some Derivatives of Acids of the Sugar Group, *Chem. Abstr.* 15: 364, 1921.
5. De Carlh, F.: Quantitative Estimation of Gluconic Acid, *Chem. Abstr.* 26: 1544, 1932.
6. Lütz, O., and Jirgensons, B.: A Simple Method for Assignment of Optically Active Hydroxy Acids to the d- and l- Series, *Ber. d. deutsch. chem. Gesellsch.* 65b: 784, 1932.
7. Schmidt, O., and Weber-Molster, C.: Sugars With a Branched Carbon Chain, *Ann. d. Chem.* 515: 65, 1934.
8. Bennet-Clark, T. A.: Polarimetric Determination of Gluconic Acid, *Biochem. J.* 28: 45, 1934.
9. Demair, W., Bleyer, B., and Schneider, L.: Gluconic Acid With Special Reference to Its Identification and Estimation, *Chem. Abstr.* 29: 4292, 1935.
10. Bodon, G.: Gluconic Acid and Hexamethylenetetramine as Urinary Tract Disinfectants, *Orvosi hetil.* 80: 845, 1936.

INVESTIGATION OF PROTECTIVE ANTIBODIES PRODUCED BY ORAL ADMINISTRATION OF TYPHOID VACCINE*

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THIS investigation was carried out with the cooperation of the Army Medical School. Colonel J. F. Siler outlined the procedure to be followed and the laboratory work was carried out under the direction of Lieutenant Colonel G. C. Dunham.

The Bacteriology Department of the University of Oklahoma carried out the following steps in the investigation:

1. Twenty-five adult individuals (20 females, 5 males) who gave a negative history for typhoid fever and for antityphoid vaccination were tested.
2. Twenty cubic centimeters of blood were secured from each.
3. The sera were separated and an agglutination titration was run on each serum.
4. The remainder of the sera was then carefully packed in sterile vaccine bottles under strict aseptic precautions and shipped to Lieutenant Colonel G. C. Dunham at the Army Medical School in Washington, D. C.
5. Here under his supervision mouse protection tests were carried out.

Following these preliminary agglutination titrations carried out in our laboratory and the titration of the protective antibodies by Lieutenant Colonel Dunham, each of the 25 individuals was given a course of oral typhoid vaccine consisting of three capsules taken at twenty-four-hour intervals, and containing in each capsule, ten thousand million heat-killed typhoid bacilli, Panama Carrier Strain No. 58. One capsule was taken on three successive mornings, one hour before breakfast.

Two and six weeks after the oral administration each individual's blood serum was titrated in our laboratory for agglutinin antibodies and samples were forwarded to Lieutenant Colonel Dunham in Washington, where they were tested for protection antibodies by the mouse protection tests. The results of these tests appear in Tables I and II.

These results confirm those previously published by us and others regarding the agglutinin antibody content in the blood of individuals orally vaccinated for typhoid fever (Hoffstadt and Thompson,¹ Hoffstadt and Martin,² Pijper and Dan,³ Simons,⁴ Crimm and Short⁵).

The results of the mouse protection tests for protective antibodies in the blood sera of the orally vaccinated individuals were practically 100 per cent negative.

The slight protection given the mice by the blood sera of three of the volunteers can be explained on the basis of experimental error, or it might be due to the "anamnestic" phenomenon.

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In a previous publication⁶ we pointed out the advisability of testing the sera of orally vaccinated people by the mouse protection method, as well as trying chemical killing of the vaccine instead of heat killing.

TABLE I
AGGLUTINATION TESTS—ORAL VACCINATIONS

INDIVIDUAL	AGGLUTININS BEFORE VACCINATION	AGGLUTININS 2 WEEKS AFTER VACCINATION	AGGLUTININS 6 WEEKS AFTER VACCINATION
1. A. B.	No agglutination 1-20	Partial agglutination 1-80	Partial agglutination 1-320
2. E. B.	No agglutination 1-20	Partial agglutination 1-80	Partial agglutination 1-320
3. J. C.	No agglutination 1-20	Partial agglutination 1-80	Complete agglutination 1-160
4. M. E.	No agglutination 1-20	Partial agglutination 1-80	No agglutination 1-320 Partial agglutination 1-320
5. J. M.	No agglutination 1-20	Partial agglutination 1-80	Complete agglutination 1-160
6. K. S.	No agglutination 1-20	Complete agglutination 1-80	No agglutination 1-320 Partial agglutination 1-320
7. M. J. M.	No agglutination 1-20	Complete agglutination 1-80	Partial agglutination 1-320
8. L. M. F.	No agglutination 1-20	Partial agglutination 1-80	Complete agglutination 1-160
9. P. L.	No agglutination 1-20	Partial agglutination 1-80	1-320
10. Mrs. J. H.	No agglutination 1-20	Partial agglutination 1-80	Complete agglutination 1-160
11. C. E. L.	No agglutination 1-20	Complete agglutination 1-80	No agglutination 1-320 No specimen obtained
12. A. W.	No agglutination 1-20	Complete agglutination 1-80	Partial agglutination 1-320
13. L. T.	No agglutination 1-20	Complete agglutination 1-80	Partial agglutination 1-320
14. F. S.	No agglutination 1-20	Partial agglutination 1-80	Partial agglutination 1-320
15. R. C.	No agglutination 1-20	Partial agglutination 1-80	Partial agglutination 1-320
16. H. M. C.	No agglutination 1-20	Partial agglutination 1-80	Partial agglutination 1-320
17. V. K.	No agglutination 1-20	Partial agglutination 1-80	No specimen obtained
18. Mrs. McM.	Partial agglutination 1-20	Complete agglutination 1-80	Complete agglutination 1-320
19. G. N.	No agglutination 1-20	Partial agglutination 1-80	Partial agglutination 1-640
20. D. S.	No agglutination 1-20	Partial agglutination 1-80	Partial agglutination 1-320
21. B. T.	No agglutination 1-20	Partial agglutination 1-80	Complete agglutination 1-320
22. K. E.	No agglutination 1-20	Partial agglutination 1-80	No agglutination 1-640 Partial agglutination 1-320
23. D. P.	No agglutination 1-20	Partial agglutination 1-80	Complete agglutination 1-160
24. L. S.	No agglutination 1-20	Partial agglutination 1-80	No agglutination 1-320 Partial agglutination 1-320
25. B. B.	No agglutination 1-20	Partial agglutination 1-80	Partial agglutination 1-320

Vaccines killed with formalin have been tested in the Army Medical School Laboratory by Siler and Dunham,⁷ using the mouse protection tests. The results of these tests showed practically no advantage in chemically killing the vaccine over the heat killing.

TABLE II
SERUM PROTECTION TESTS OF ORALLY VACCINATED INDIVIDUALS

SERUM NO.	INDIVIDUAL	BEFORE VACCINATION	NO. OF M.L.D.S AGAINST WHICH 0.1 C.C. AMOUNTS OF SERUM PROTECTED ALL MICE	
			2 WEEKS AFTER VACCINATION	6 WEEKS AFTER VACCINATION
0-1	A. B.	- 1	- 1	- 1
0-2	E. B.	1	1	- 1
0-3	J. C.	- 1	- 1	1
0-4	M. E.	1	- 1	- 1
0-5	J. M.	- 1	- 1	- 1
0-6	K. S.	- 1	- 1	1
0-7	M. J. M.	1	- 1	- 1
0-8	L. M. F.	1	1	1
0-9	C. E. L.	- 1	1	
0-10	Mrs. J. H.	- 1	- 1	- 1
0-11	P. L.	10	100	1
0-12	A. W.	10	10	1
0-13	L. T.	- 1	- 1	- 1
0-14	F. S.	- 1	- 1	- 1
0-15	R. C.	- 1	- 1	1
0-16	H. M. C.	- 1	- 1	- 1
0-17	V. K.	- 1	- 1	---
0-18	Mrs. McM.	1	- 1	- 1
0-19	G. N.	- 1	- 1	- 1
0-20	D. S.	- 1	1	1
0-21	B. T.	- 1	1	- 1
0-22	K. E.	1	1	10
0-23	D. P.	- 1	- 1	1
0-24	L. S.	- 1	1	- 1
0-25	B. B.	- 1	- 1	10

In our previous experiments we did not test for the H agglutinins because we felt at the time that heat killing the vaccine destroyed the H antigen. Crimm and Short, using the same strain of *B. typhosus* in preparing typhoid vaccine for oral administration, and orally administering it in the same way we did, have been able to demonstrate the presence of H agglutinin antibodies for *B. typhosus* in the blood sera of people orally vaccinated.

The combined results of practically all the experiments with oral administration of typhoid vaccine to date show that agglutinin antibodies both O and H for *B. typhosus* are found in the blood sera of individuals following the vaccination.

Agglutinin, precipitin, and lytic antibodies for *B. typhosus* have been demonstrated in the blood sera of people orally vaccinated for typhoid by Hoffstadt, Thompson, and Martin. Protective antibodies as revealed by the mouse protection tests at the Army Medical School, Washington, D. C., failed to show their presence in any significant quantity. Various field trials with oral typhoid vaccine in South America, Africa, and Europe have had encouraging results. It is, therefore, reasonable to state that the oral administration of typhoid vaccine may possibly be an effective method, but at the present time it is not advisable to accept it or to recommend it.

The intradermal method reported by Siler and Dunham⁸ at the Army Medical School and Tuft, Yagle, and Rogers^{9, 10} at the Temple University School of Medicine, furnishes definite evidence that it is effective. It also practically eliminates the most objectionable feature of the subcutaneous administration of typhoid vaccine, namely, the general incapacitating systemic reaction.

REFERENCES

1. Hoffstadt, Rachel E., and Thompson, Randall L.: Immunological Studies of Typhoid Vaccination by Mouth. II. Complement Fixations and Precipitin Reactions in Persons Treated Orally With Bile and Triple Typhoid Bacterin, *Am. J. Hyg.* 9: 21, 1929.
2. Hoffstadt, Rachel E., and Martin, Carl L.: Immunological Studies of Typhoid Vaccination by Mouth. III. Agglutinins and Complement Fixation Reactions Formed in Persons Treated With Typhoid Capsules, *Am. J. Hyg.* 9: 37, 1929.
3. Pijper, Adrianus, and Dan, Helen: Typhoid Agglutination After Oral Immunization, *Brit. J. Exper. Path.* 11: 112, 1930.
4. Simons, Joseph: Absorption of Antigens From Body Surfaces, *Proc. Soc. Exper. Biol. & Med.* 29: 20, 1931.
5. Crimm, Paul D., and Short, Darwin M.: A Study of Oral Typhoid Vaccination as Measured by Blood Serum Agglutinins, *Am. J. M. Sc.* 196: 826, 1938.
6. Moor, H. D., and Brown, Ida L.: A Comparative Study of Oral and Subcutaneous Vaccination Against Typhoid Fever Based on Agglutination Titrations, *J. Lab. & Clin. Med.* 22: 1216, 1937.
7. Siler, J. F., Colonel, and Dunham, G. C., Major: Duration of Immunity Conferred by Typhoid Vaccination. Results of Revaccination by the Intracutaneous Injection of Typhoid Vaccine, *Am. J. Pub. Health* 29: 95, 1939.
8. Siler, J. F., Colonel, and Dunham, G. C., Major, and others: Protective Antibodies in Blood Serum of Individuals After Immunization With Typhoid Vaccine, *Am. J. Pub. Health* 27: 142, 1937.
9. Tuft, Louis, Yagle, E. M., and Rogers, Stuart: Comparative Study of Antibody Response After Various Methods of Administration of Mixed Typhoid Vaccine With Particular Reference to Intradermal and Oral Methods, *J. Infect. Dis.* 50: 98, 1932.
10. Tuft, Louis: Further Studies of Intracutaneous Method of Typhoid Vaccination, *Am. J. M. Sc.* 199: 84, 1940.

COMPLEMENT FIXATION WITH SPECIFIC ALLERGENS IN HAY FEVER

I. PROBLEM, PROCEDURE, DIAGNOSTIC VALUE

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IT IS still a moot question whether the treatment of hay fever and asthma with specific antigens immunizes or de-immunizes (desensitizes, de-allergizes). Different methods (skin tests, conjunctival tests, Prausnitz-Küstner reaction) gave highly contradictory results to different investigators.

The first theory offered is that of Noon,¹ who considered the offending protein as a toxin against which the body forms an antitoxin. Noon, therefore, aimed at active immunization and tried to increase the antitoxin titer in the tissues, using the conjunctival test as a means of checking his theory. After each therapeutic inoculation he found a decrease in the degree of conjunctival reaction during the first few days, followed by a rise to a higher level if a

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proper amount of "toxin" had been given. Noon's findings with regard to the negative phase and the following peak of immunity correspond to those of Brieger and Ehrlich,² and of Wright³ in their experiments with bacterial vaccines. Freeman⁴ and Cooke⁵ adopted this view. Pasteur-Vallery-Radot, and Haguénau,⁶ however, obtained opposite results by a different method of treatment. They gave daily intracutaneous injections to an asthmatic patient sensitive to horses and observed that the patient's diminishing susceptibility to the presence of horses was accompanied by a decrease of his skin reactions, which finally disappeared entirely. Cooke,⁵ on the other hand, did not see any decrease of skin reactions during specific treatment, whether the patients were benefited or not. Levine and Coca⁷ investigated the serum of allergic patients by means of the Prausnitz-Küstner reaction (passive transfer). They found that during a short course of pre-seasonal treatment the reactions decreased in some cases, and increased in others. They conclude that their study furnished no proof that the effect of specific treatment of atopic conditions is due to desensitization. Colmes and Rackemann⁸ state that the success of preseasonal specific treatment is independent of the degree of the skin reaction at the end of the course, and that the degree of skin reaction is not related to the total dose given. Black⁹ emphasizes that the question as to what is being done by specific treatment is still open to investigation. Baldwin and Glaser¹⁰ state that there is no correlation between skin reactivity and the success of treatment and decrease of the reactivity of the mucous membranes. The passive transfer gives inconclusive results.

An entirely different method of approach was employed by Walker¹¹ and Albus¹² who used complement fixation tests.

Walker studied a group of 30 patients who by history and skin tests were sensitive to one or several of the following proteins: horse dander, cat hair, wheat, and *Staphylococcus aureus*. The complement fixation with the offending proteins was positive in some patients, negative in others. In all patients who had a positive complement fixation, this was decreased by specific treatment. A negative complement fixation would stay negative throughout the treatment. Nonallergic persons had negative complement fixation. Albus studied 8 hay fever patients who had a positive complement fixation with those pollens (grasses and trees) to which they were sensitive according to history and skin tests. The complement fixation was found negative after a course of pre-seasonal treatment sufficient to relieve the symptoms. Albus claims that the necessity of treatment in the following year may be judged by the result of a complement fixation test performed before treatment is begun.

Contrary to the other methods mentioned, the complement fixation test gave consistent results to those who used it, and appeared to offer an opportunity to study in detail the patient's reactivity during the different phases of specific treatment. We decided, therefore, to adapt Albus' methods to the conditions of the Midwestern States, and to repeat his study on a larger number of patients, and in more extensive fashion.

PROCEDURE

Albus' method is the complement fixation test as modified by Hecht.¹³ It was first applied to allergic patients by Jaffe.¹⁴ Active human serum and un-

sensitized sheep cells form the hemolytic system. Inhibition, in the presence of an antigen, of the normal hemolytic power of the active serum indicates the presence of specific antibodies and is considered a positive test. The method requires determination of the hemolytic titer of each individual serum before the actual test is performed.

Technique.—For the preliminary reaction 0.2 c.c. of the serum and 1.0 c.c. of normal saline were placed into each of ten Kahn tubes and incubated at 37° C. for thirty minutes. Then graded amounts of a 5 per cent suspension of freshly washed sheep blood corpuscles were added, the gradations beginning with 0.1 c.c. of the suspension in the first tube and successively increased in each following tube so that the last tube receives fully 1.0 c.c. of red blood cells. The tubes were incubated once more at 37° C. for thirty minutes, and the reaction was read at the end of this time. The highest amount of sheep cells, which had become completely hemolyzed, was recorded. The procedure for the actual test was similar, but the normal saline was replaced by solutions of the antigen under investigation. One cubic centimeter of graded antigen dilutions was placed into each tube with 0.2 c.c. of serum and incubated for half an hour. After this time a proper amount of sheep cells, as determined in the preliminary test, was added to each tube, and the mixture was incubated once more for thirty minutes. The reaction was read at the end of this time, and again after being placed in the icebox for twenty-four hours. The highest dilution of antigen is recorded which shows nonhemolyzed sheep cells.

Antigens.—Extracts of the dried pollens of short ragweed, orchard grass, and June grass were used as antigens. Normal saline, rather than Coca's solution,¹⁵ was used as the extracting fluid because the presence of a preservative might influence the test. A weighed amount of the pollen was extracted with 100 c.c. of normal saline (for intravenous use) in the icebox at 5° C. for forty-eight hours. The fluid was then filtered through a Seitz filter, and was ready for use if proved sterile in aerobic and anaerobic cultures. Two different extracts were prepared of each species of pollen. An extract of 5 Gm. of pollen in 100 c.c. of saline was called "concentrated," while an extract of 1 Gm. in 100 c.c. was used as "stock fluid" (dilution No. 0). From this solution progressive dilutions were obtained by mixing a measured quantity with an equal amount of normal saline and repeating this procedure with part of the diluted solution. The antigen solutions in Table I were used.

TABLE I

CONCENTRATION	DESIGNATION
5:100	Concentrated
1:100	Stock fluid (No. 0)
1:200	Dilution No. 1
1:400	Dilution No. 2
1:800	Dilution No. 4
1:1,600	Dilution No. 8
1:3,200	Dilution No. 16
1:6,400	Dilution No. 32

EXPERIMENTAL WORK

a. *Controls.* In order to test the specificity of the reaction, and at the same time determine the anticomplementary properties of the pollen extracts,

TABLE II

RESULTS OF COMPLEMENT FIXATION TESTS ON UNRELATED HAY FEVER PATIENTS AND CONTROLS

	COMPLETELY NEGATIVE	POSITIVE UP TO DILUTION								TOTAL TESTS
		CONC.	NO. 0	NO. 1	NO. 2	NO. 4	NO. 8	NO. 16	NO. 32	
Ragweed	Unspecific range				Specific range					25
Controls	8	4	8	5	—	—	—	—	—	
Patients	1	—	3	2	3	2	2	2	6	27
Orchard Grass	Unspecific range				Specific range					24
Controls	13	2	9	—	—	—	—	—	—	
Patients	1	1	3	1	2	—	1	1	2	14
June grass	Unspecific range				Specific range					22
Controls	16	5	1	—	—	—	—	—	—	
Patients	—	1	5	2	2	—	—	1	2	13

a number of persons in whom pollen sensitization could be excluded were used as controls (Table II).

Twenty-five sera of normal individuals and of allergic patients not sensitive to pollens were tested with ragweed antigen. While some of the sera showed inhibition of hemolysis (complement fixation) with high concentrations of this extract, none reacted with any dilution higher than No. 1 (1:200). On this basis complement fixation with No. 2 and higher dilutions of ragweed extract was considered specific in our later work. Twenty-four of these sera were also tested with orchard grass antigen. Sixteen sera of the same group and six sera of hay fever patients not sensitive to June grass were tested with June grass antigens. Reactions occurred only with concentrated and stock fluids. Therefore, complement fixation with No. 1 and higher dilutions of orchard grass and June grass extracts was considered specific.

b. *Hay Fever and Asthma Cases.*—The sera of 51 patients who had, or had had, symptoms of pollinosis were examined by the complement fixation method. All these patients had been found sensitive to one or more of the pollens by both history and scratch tests. Many patients were examined more than once, and with few exceptions all three pollen extracts were used in each instance.

For our purposes the patients were divided into two major groups: those who had never had specific treatment or who had interrupted such treatment for a prolonged period, and those who were examined while they were receiving perennial treatment at regular intervals. The first group only will be considered in this communication.

There were 25 patients who had suffered from hay fever for from sixteen months to thirty years prior to examination. Eighteen persons of this group had never had specific treatment; 6 patients had had preseasonal and coseasonal treatment in previous years, but had not received injections for periods ranging from seven weeks to eleven months. The results of the complement fixation test were essentially similar in these two classes, and will be discussed together.

Twenty-three patients were sensitive to ragweed, 12 were sensitive to orchard grass, and 11 were sensitive to June grass. Blood for serologic examination was taken usually shortly after, occasionally before, the diagnostic scratch tests were administered. At this first examination 22 of 23 patients sensitive to ragweed showed some degree of complement fixation with ragweed antigen; 11

of 12 patients with orchard grass sensitization reacted with orchard grass extract, and all 11 patients sensitive to June grass showed inhibition of hemolysis with June grass. The general range of antibody titers was definitely higher

TABLE III

RESULTS OF SKIN TESTS AND COMPLEMENT FIXATION TESTS IN 25 UNTREATED CASES OF HAY FEVER

CASE NO.	AGE	SEX	DURATION OF HAY FEVER	SCRATCH TESTS			DATE	COMPLEMENT FIXATION TESTS			REMARKS
				SHORT RAG-WEED	ORCHARD GRASS	JUNE GRASS		SHORT RAG-WEED	ORCHARD GRASS	JUNE GRASS	
1	33	M	5 years	++++	++++	++++	7/23/37	No. 32	No. 32	No. 1	
2	59	M	26 years	++++			10/27/37	No. 32			
3	36	F	5 years	++++			11/16/37	No. 32			
4	25	M	Since childhood	++++	++++	+++	4/12/37	No. 32	No. 8	No. 32	Tree pollens +++
5	22	F	20 years	++++			3/24/37 8/23/37	No. 0 No. 32			
6	36	F	22 years	++++	++++	++++	4/ 5/37 4/ 8/37 10/29/37	No. 0 No. 4 No. 32	Conc. Conc. No. 32	No. 0 No. 0 No. 32	
7	40	F	Since childhood	++++	+++	++++	1/12/37	No. 16	No. 16	No. 0	
8	30	F	Many years	+++			11/10/37	No. 16			
9	29	F	15 months	++++			11/10/36	No. 8			
10	18	F	1 year	++++			7/ 1/37	No. 8			
11	19	M	Since childhood	++++			8/26/37	No. 4			Timothy +++
12	43	M	Since childhood	++++			10/25/37	No. 4			
13	25	M	7 years	++++	+++	+++	4/ 2/37 6/11/37	No. 1 No. 4	Conc. No. 2	Conc. No. 1	
14	21	M	Since childhood	++++	+++	+++	10/21/37	No. 2	Neg.	No. 0	
15	26	M	5 years	++			6/ 8/37	No. 2			Molds +++
16	29	M	Many years	++++			3/30/37 4/ 2/37	No. 1 No. 2			
17	17	M	First season	++++	++	++	10/27/37	No. 1	No. 0	No. 0	Molds +++
18	58	M		++++			3/ 1/37 3/ 5/37	No. 1 No. 0			
19	37	F	17 years	++++			10/22/37	No. 0			
20	23	F	5 years	++++	+++		5/ 7/37	No. 0	Conc.		
21	24	M	10 years	++++	+++	+++	10/20/37	No. 0	No. 0	No. 0	
22	28	F	24 years	++++	++	++	2/19/37	No. 0	No. 2	No. 2	
23	34	M	4 years	++++			7/ 8/37	Neg.			Timothy +++
24	25	M	Since childhood		++++	++++	4/10/37		No. 0	No. 2	
25	23	F	10 years		++++	±	5/24/37		No. 1	No. 16	

in hay fever cases than in the control group, there was a wide variation of reactivity, and only twelve, five, and five tests, respectively, were within the specific range. The amount of reagin in the serum appeared to be independent of the degree of positivity of the skin tests (Cases 4, 7, 22, 25). Neither was the age of the patient, the duration of the disease, nor the time of the year at which the blood was taken in any constant relationship to the antibody titer.

Repetition of the test, however, furnished some interesting findings. In five cases we were able to obtain another blood sample at a later date. Case 13 was re-examined two months after the first serologic test, two days after the administration of positive scratch tests. The antibody titer had increased. Cases 6, 16, and 18 were checked a few days after the first examination which, in these cases, practically coincided with the skin tests. The antibody titer for ragweed had increased in two of them; it was still lower in Case 18, a farmer who was continuously exposed to ragweed in his occupation. It is reasonable to assume that the diagnostic scratch test had brought about a partial increase of floating antibodies in those patients who were not otherwise in contact with the pollens at that particular time of the year. Case 6 was seen once more at the end of the ragweed season without having received specific treatment in the interval. This time she reacted to all three antigens in the highest dilutions. Case 5 had been first examined before the beginning of the ragweed season. Because her history was not entirely significant for ragweed sensitization, and because she had positive skin tests for molds in addition to the one for ragweed, she was started on injections with mold extracts. She spent most of the ragweed season in the northern part of Michigan and was free of symptoms. Three days after returning home she developed a severe asthmatic state which lasted for five days before she was seen at the clinic. At that time complement fixation with ragweed was strongly positive.

COMMENT

The titration of antibodies for diagnostic purposes by the complement fixation test, as it was undertaken in this study on 25 normal persons and 25 hay fever patients, shows that while a positive reaction with higher dilutions of the specific pollen extracts is found in sensitized individuals only, there is a relatively large percentage of allergic persons who have low titers of serum reagins without having had "desensitizing" treatment. The test does not appear to be suitable to replace the skin tests in the diagnosis of hay fever. It promises, however, to be a valuable tool for the investigation of the mechanism of pollinosis.

Our experiments show—in agreement with the results of previous workers—that specific antibodies can be demonstrated in the serum of hay fever patients both in and out of season. It is apparent that skin reactivity and the quantity of floating antibodies do not parallel each other, and that the serum reagins are more variable than the reactivity of the skin, a result which conforms with the experiences of protein sensitization of animals (Kahn). Cases 4, 7, 22, and particularly 25, show in addition that in the same blood sample there may be a high antibody titer for one antigen and a low titer for another; the corresponding scratch tests show just the opposite result. Fluctuations of reagin titer occurred in three of four cases following a diagnostic scratch test. This makes it likely that had the examination been repeated in other instances, posi-

tive complement fixation would have been found in a much larger percentage than our tables suggest. The alteration of the antibody titer following a scratch test tends to emphasize two advantages that a serologic examination has over a skin or mucous membrane test in the investigation of the allergic mechanism. The serologic reaction enables us to test the amount of antibodies available in the body at any precise moment, and it does not influence the allergic state of the patient. The administration of even a minute quantity of the allergen to one of the body surfaces, however, causes the organism to react, and will in itself influence the result of the next examination.

Case 4 suggests, on the other hand, that the antibodies which are demonstrated by the complement fixation test are not identical with the reagins responsible for the Prausnitz-Küstner reaction. In this case a blood sample taken three days after the scratch test showed a positive complement fixation with ragweed, low titers for orchard grass and June grass. A passive transfer performed with the same serum was positive for all three allergens.

SUMMARY

Hecht's modification of the complement fixation test was used to determine the amount of specific antibodies in the blood of patients suffering from hay fever. Extracts of the pollens of short ragweed, orchard grass, and June grass were used as antigens.

Twenty-five normal controls showed only occasional reactions with highly concentrated extracts. Twenty-five hay fever patients who either had received no specific treatment at all, or had received no treatment recently, showed reactions with much higher dilutions of the allergens, particularly if examined repeatedly under varied conditions.

The significance of fluctuations of the antibody titer has been discussed.

REFERENCES

1. Noon, L.: Prophylactic Inoculation Against Hay Fever, *Lancet* 1: 1572, 1911.
2. Brieger, L., and Ehrlich, P.: Beiträge zur Kenntnis der Milch immunisierter Tiere, *Ztschr. f. Hyg. u. Infektionskr.* 13: 336, 1893.
3. (a) Wright, A. E.: On Therapeutic Inoculations of Bacterial Vaccines; and Their Practical Exploitation in the Treatment of Disease, *Brit. M. J.* 1: 1069, 1903.
(b) Wright, A. E., and Douglas, S. R.: On the Action Exerted Upon the Staphylococcus pyogenes by Human Blood Fluids, and on the Elaboration of Protective Elements in the Human Organism in Response to Inoculations of a Staphylococcus Vaccine, *Proc. Roy. Soc., London*, s.B. 74: 147, 1904-5.
4. Freeman, J.: Further Observations on the Treatment of Hay Fever by Hypodermic Inoculations of Pollen Vaccine, *Lancet* 2: 814, 1911.
5. (a) Cooke, R. A.: The Treatment of Hay Fever by Active Immunization, *Laryngoscope* 25: 108, 1915.
(b) Cooke, R. A., and Vander Veer, A.: Human Sensitization, *J. Immunol.* 1: 201, 1916.
6. Pasteur-Vallery-Radot, P., and Huguennau, J.: Asthme d'origine équine, *Bull. Soc. méd. d. Hôp. de Paris* 45: 1251, 1921.
7. Levine, P., and Coca, A. F.: Studies in Hypersensitiveness; XXII Nature of Allerviating Effect of Specific Treatment of Atopic Conditions, *J. Immunol.* 11: 449, 1926.
8. (a) Colmes, A.: Serial Skin Tests as Guide in Treatment of hay Fever, *Preliminary Report, J. Allergy* 3: 449, 1932.
(b) Colmes, A., and Rackemann, F. M.: Further Observations on Changes in Skin Tests Following Specific Pollen Therapy, *J. Allergy* 4: 473, 1933.
9. Black, J. H.: Some Pressing Problems in Allergy, *J. A. M. A.* 99: 1, 1932.
10. Baldwin, L. B., and Glaser, J.: Effect of Treatment on Skin and Mucous Membrane Sensitivity and on Reagins in Hay Fever, *J. Allergy* 8: 129, 1937.

11. Walker, I. C.: Study XII. Complement Fixation and Precipitin Reactions With the Serum of Bronchial Asthmatics Who Are Sensitive to the Proteins of Wheat, Horse Dandruff, Cat Hair and Bacteria, Using These Proteins as Antigens and the Cutaneous Reactions as an Index of Sensitization, *J. M. Research* 36: 243, 1917.
12. (a) Albus, G.: Beeinflussung der Antikörperkonzentration durch spezifische Desensibilisierung bei Pollenallergie, *Ztschr. f. d. ges. exper. Med.* 95: 703, 1935.
 (b) Albus, G.: Serologie der Pollenkrankheit unter Berücksichtigung des Antikörpers, *Med.* 96: 710, 1935.
 (c) Albus, G.: Über die Bedeutung des Antikörpers bei Behandlung der Pollenallergie, *Ztschr. f. d. ges. exper. Med.* 97: 756, 1936.
13. Cited in (a) Kolle, W., and Hetsch, H.: *Die experimentelle Bacteriologie und die Infektionskrankheiten*, ed. 5, Berlin, 1919, Urban and Schwarzenberg, 1, 240.
 (b) Jadasohn, J.: *Handb. d. Haut- u. Geschlechtskr., Serologie der Syphilis*, Berlin, 1929, Julius Springer.
14. Jaffé, K.: Nachweis spezifischer Antikörper in vitro bei hochgradiger Fisch- und Hefeallergie, *Klin. Wchnschr.* 10: 304, 1931.
15. Grove, E. F., and Coca, A. F.: Studies in Specific Hypersensitiveness; XV Nature of Atopens of Pollens, House Dust, Horse Dander and Green Pea, *J. Immunol.* 10: 471, 1925.
16. Kahn, R.: *Tissue Immunity*, Springfield, Ill., 1936, Charles C Thomas.

AN EVALUATION OF THE IMPORTANCE OF FUNGI IN RESPIRATORY ALLERGY

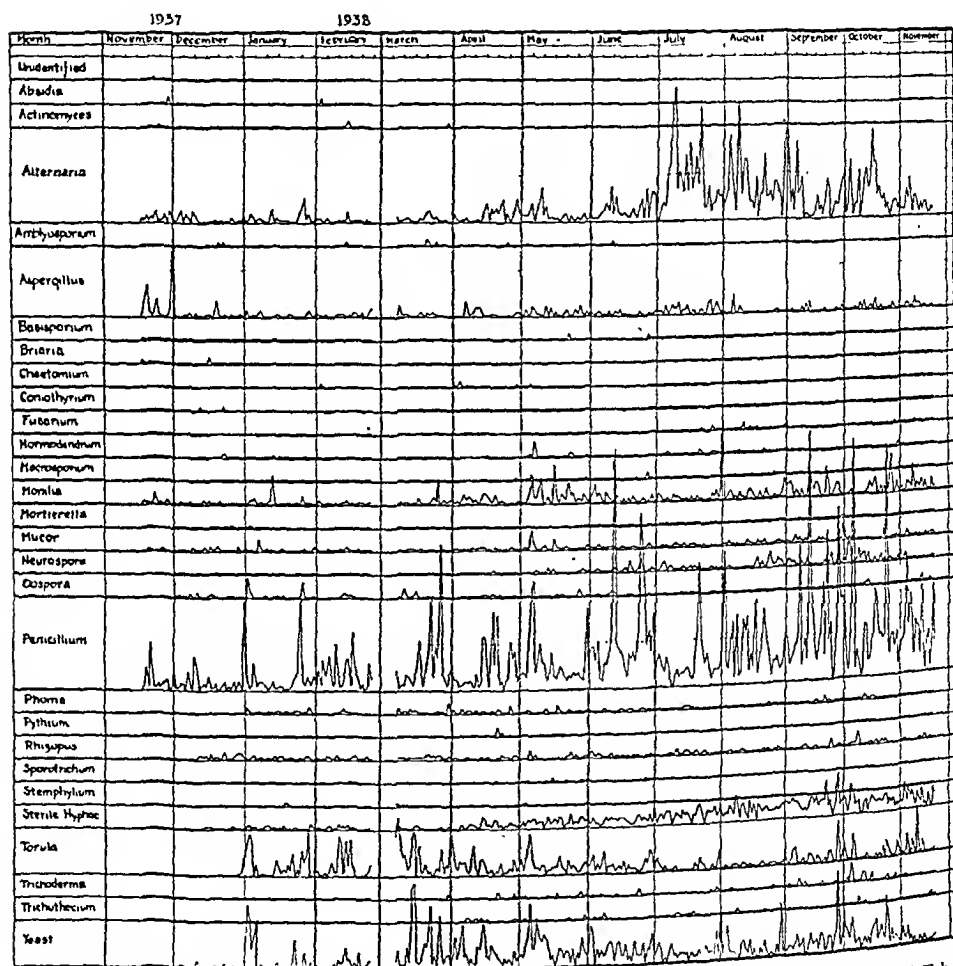
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CONSIDERABLE attention has been focused recently upon the relation of fungus spores to allergic diseases of the respiratory tract. At times large quantities of wind-borne spores may be present in the air, fungus seasons similar to pollen seasons have been noted; skin tests to fungi are often positive. However, the question as to how much symptoms of respiratory allergy are actually due to molds is still highly controversial. During the past three years we carried out, in collaboration with Ascher,¹ a comprehensive study of this question which included both an air survey and a clinical follow-up study.

AIR SURVEY

A brief review of the air survey is necessary for the present report. One of us (A. B. A.) exposed Sabonraud plates each day for the period of one year. Proper controls were made throughout the duration of the survey by exposure of plates at the same time and under the same experimental conditions 3 feet and 90 miles apart. Other controls included simultaneous exposures at different heights and in twenty-three different habitats for fungi throughout the city, such as in a bakery, butcher shop, pigpen, chicken coop, and barnyard. Clear patterns for the presence of certain fungi were recognized which tended to tally in all these different exposures on certain days and during certain seasons. Individual differences, however, were very pronounced. Precipitation and low barometric pressure tended to free the air temporarily from fungus spores. Inland winds, as well as high pressure, increased the count. Continued

freezing and subfreezing weather inhibited propagation of spores. Among the perennial fungi (Graph 1) *Penicillium*, yeast, and *Torula* produced the greatest number of colonies. *Alternaria* and *Monilia*, while present throughout the year, showed a distinct seasonal increase, namely, *Alternaria* from July to November and *Monilia* from May to November. Another seasonal increase was observed for yeasts and *Torula* in the early part of the year. In June and early July rust was found on slides which we exposed simultaneously with the culture plates, and smut occurred from July through October.



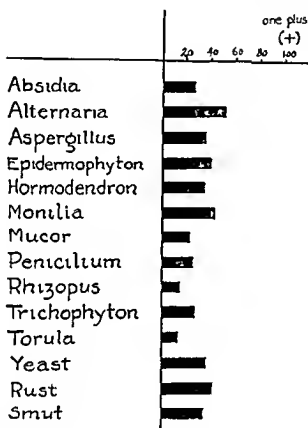
Graph 1.—Air survey. Scale— $\frac{1}{4}$ inch equals 100 colonies. (No count from latter part of February to first part of March due to illness of A. B. A.)

METHODS

In this study the following methods were employed: (1) Intradermal skin tests with fungus extracts. (2) Fungus cultures and microscopic examination of smears from nasal and bronchial secretions. (3) Symptom "calendars" for each patient during the period of the fungus survey. (4) Exposures of fungus plates in patients' homes. We refrained from making extensive passive transfer studies because we found these tests less conclusive than skin tests.

SKIN REACTIONS

Comparison of Individual Fungi.—With the thirteen genera of fungi* most frequently encountered in the air survey, intradermal skin tests were performed on 841 patients with upper respiratory allergy. The dry extracts were prepared according to the method of Feinberg.² The average reactions determined for each fungus per patient are shown in Graph 2.† A total of 580 patients, or 69 per cent of the entire group, gave positive reactions to one or more fungi. Alternaria, Monilia, Epidermophyton, and rust predominated slightly in skin reactivity over the others. Torula and Rhizopus gave the weakest reactions.



Graph 2—Average skin reaction (+ to +++) for each fungus (841 patients).

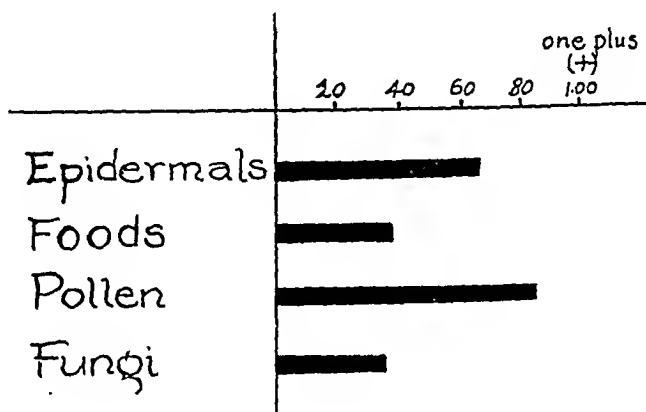
Comparison in Different Types of Cases.—On the basis of history and clinical behavior the 841 cases were subdivided into the following groups: (1) Patients with seasonal hay fever (267 cases) and asthma (182) due to pollen. (2) Nasal allergy (110 cases) and asthma (219) occurring seasonally but not accounted for by pollen. (3) Perennial upper respiratory allergy, namely, 38 cases of asthma and 25 of vasomotor rhinitis. (4) In addition to these three groups with a total of 841 patients, 39 cases were included for comparative purposes, namely, 28 cases of chronic sinus infection and 11 cases of bronchiectasis. In these patients an allergic basis for the infectious state could not be definitely established. No noteworthy data were detected by thus subdividing our cases. Especially in the second group of seasonal "nonpollen" allergy in which fungi were suspected to be major causative agents, the skin reactions coincided well with those of the other patients. Those of the first group, primarily sensitive to

*According to Jadassohn and others (J. Immunol. 32: 203, 1937), the individual species of a fungus behave antigenically like the others of the same genus. We, therefore, refrained from identification of the species.

†They were obtained by adding all the plus marks in all patients for the respective organism and dividing the total by the total number of patients.

pollen, gave stronger positive reactions to fungi than those in the second and third groups. To our surprise, reactions encountered were by far stronger among the cases with chronic sinusitis than in any other patients.

Comparison With Other Antigens.—In an attempt to compare the total number of skin reactions obtained for fungi with those for other antigens in the 841 patients, Graph 3 was established.* If measured by the standard of skin reactivity, fungi are considerably less important than pollens and epidermals but about equally as important as foods.



Graph 3.—Comparison of reactions to fungi with those of other antigens.

Constancy.—Skin reactions to pollen are known to remain positive for a long time. The minor reactions to foods and epidermals are subject to great variations while the majority of the three- and four-plus reactions to foods remain rather stable (Waldbott and Aseher³). Eighty-one of our patients were tested twice and 15 were tested three times for the same fungi. Only a few of the three- and four-plus reactions remained positive upon subsequent testing. The minor reactions tallied only very rarely with those of the original tests.

CULTURES FROM RESPIRATORY SECRETIONS

Of 442 cultures grown on potato dextrose agar medium of a pH 5.5 (222 from nasal secretions, 220 from bronchial secretions) 26 showed no growth, 18 showed sterile hyphae only, and 124 developed bacterial growth. In 274 cultures fungi were grown. *Penicillium* predominated in 122, then followed *Alternaria* (62), *Monilia* (48), and yeast (42). *Aspergillus*, *Hormodendrum*, and *Torula* were less prevalent. The heaviest growths occurred in October and November. *Hormodendrum*, *Alternaria*, and *Aspergillus* were present only from July through November. In the majority of cases we did not consider positive sputum cultures diagnostic, but merely incidental. It is likely to occur in practically any respiratory secretion. In only 5 patients was the same fungus grown twice. In 9 of the 442 specimens the microscopic examination with and without staining revealed mycelia which we were unable to identify. *Alternaria* spores were found in 3, *Monilia* in 3, and yeast in one.

*Since in our routine testing many more tests for foods are made than for pollen, epidermals, and fungi, we had to put the results on a comparative basis: The total number of plus marks in all patients for one group of antigens was divided by the number of antigens in this group.

SYMPTOM CALENDARS

At the outset of our study every patient was provided with a printed form on which he was to record the degree (one plus to four plus) as well as the dates of his symptoms throughout the year. For several months we checked rather carefully the occurrence of the symptoms with each peak of our fungus curve. When there was a general increase of the fungus content in the air, for instance in late October and November, most patients showed a more or less pronounced tendency to be worse. This we were not able to attribute to one specific fungus, nor did patients react to the majority of fungi encountered in the air on those days. With altered weather conditions, especially strong winds and high barometric pressure, many solid particles of organic and inorganic nature, such as dust, soot, insect parts, were present on the exposed slides. They could have provided these symptoms as well as fungi. The only definite causative agents established by this method were rust and smut.* This is set forth in another publication (Waldbott and Ascher⁴). There were 7 patients who exhibited symptoms only at the height of the rust and smut season of 1938, and 12 who suffered marked exacerbation at the time.

HOME SURVEYS

Patients who suspected factors in their home surroundings as a cause of their allergic symptoms exposed Sabouraud plates in their homes for a quarter of an hour on several occasions. Without exception these plates showed the usual growth of fungi, but no abnormal predominance of a single species could be found. These patients were retested after the identification of the growth on the plate, and treated if the respective fungi produced positive reactions. Again no clear-cut relationship was established between the fungus encountered in the patient's home and his symptoms. This is in line with the fact that, as mentioned above, in simultaneous exposures at 23 different habitats no qualitative difference in the growth was noted.

DISCUSSION

Evidently the prevalence of a certain fungus in the air does not necessarily coincide with its actual clinical significance. Some fungi exhibit greater antigenicity than others. *Alternaria*, for instance, as was also pointed out by Feinberg⁵ and Pratt,⁶ gave the strongest skin reactions. *Penicillium*, which outnumbered all others in the cultures recovered from the air and mucous secretions, was considerably less important clinically. *Epidermophyton*, which grew very scantily on our culture plates, ranked foremost among the fungi as far as skin reactivity is concerned.

In explanation of the more pronounced antigenic activity several possibilities may be offered. Most of the fungus spores giving the strongest skin reactions are larger in size than the weaker reacting ones. Rust and most smuts are spiculated, thus being local irritants when in contact with the mucous membranes. These latter fungi show a seasonal appearance and partial or complete

*Rust and smut do not grow on Sabouraud plates. Their presence was determined by a 1% survey.

absence from the air during the balance of the year. There is usually a sudden increase in their concentration when they first appear in the air. All these features are known to account for more pronounced symptoms with pollen.

True respiratory sensitization due to one single fungus was encountered in only a few cases. This is in agreement with the scarcity of similar reports in the literature. But fungus infections (mycosis), which may or may not be associated with allergy, were found in a considerable number of cases. Since they cannot always be distinguished from true allergic asthma and sinus diseases, their incidence in this series cannot be given. The patients exhibit such symptoms as wheezing, coughing, nasal and sinus catarrhs which are often seasonal, usually occurring in late fall. Episodes of low-grade fever may occur. A family background of allergy and eosinophilia in blood and respiratory secretions may or may not be present. Skin tests for other antigens are negative. The responsible fungus gives positive skin reactions, the degree of which varies with the different stages of the disease. Repeated detection of the same fungus in the cultures is the chief diagnostic criteria. Occasionally the x-ray appearance of the chest may permit the diagnosis, there being an excessive enlargement of the hilum shadows and small sharply delineated focal lesions, especially in the lower portions of the lungs which gives the lungs a peculiarly mottled appearance. Occasionally there are fungus lesions on the skin, especially in the body folds, from which the organisms can be grown. Oral and intravenous administration of iodides may be of diagnostic value since it often affords prompt clinical improvement in certain fungus infections.

SUMMARY

1. The role played by fungi in respiratory allergy (asthma and allergic nasal disease) was investigated by an atmospheric survey of fungi, and a clinical study of 841 patients with upper respiratory allergy was made. This study covered intradermal skin tests, fungus cultures, and microscopic examinations of nasal and bronchial secretions, a follow-up of the dates of the patients' symptoms by means of "symptom calendars," and fungus surveys in the patients' homes.

2. Skin reactions to one or more fungi were positive in 69 per cent of the 841 allergic patients. Fewer positive reactions were obtained to fungi than to pollens and epidermals, but about as many as to foods. The strongest positive reactions to fungi occurred in patients who also reacted strongly positive to other antigens.

3. In the cultures from nasal and bronchial secretions, *Penicillium*, *Alternaria*, *Monilia*, and yeast prevailed. During October and November fungi grew more abundantly from the respiratory secretions than at any other period.

4. Plates exposed in patients' homes revealed no characteristic habitats of fungi in any single case.

5. The follow-up of the dates of symptoms indicates that respiratory allergy due to one single fungus is very rare. More often fungi are complicating factors in multiple sensitive patients with asthma and vasomotor rhinitis. During the summer months the most common offenders are smut, *Alternaria*, and rust. As

such they should be given special consideration in the treatment of hay fever. *Penicillium*, which was by far the most common fungus found in the atmosphere, homes, and mucous secretions, was of relatively minor significance clinically.

6. Some of our cases were found to simulate merely asthma and vasomotor rhinitis. They represented true fungus infections. The diagnostic criteria in these cases are outlined.

REFERENCES

1. Waldbott, G. L., and Ascher, M. S., and Ackley, A. B.: Air Survey in Southern Michigan, *J. Michigan Med. Soc.* 39: 645, 1940.
2. Feinberg, S. M.: Mold Allergy, Its Importance in Asthma and Hay Fever, *Wisconsin M. J.* 34: 251, 1935.
3. Waldbott, G. L., and Ascher, M. S.: Serial Skin Reactions to Foods, Unpublished.
4. Waldbott, G. L., and Ascher, M. S.: Rust and Smut—Major Causes of Respiratory Allergy, *Ann. Int. Med.* 14: 215, 1940.
5. Feinberg, S. M.: Seasonal Hay Fever and Asthma Due to Molds, *J. A. M. A.* 107: 1861, 1936.
6. Pratt, H. N.: Seasonal Aspects of Asthma and Hay Fever in New England, *New England J. Med.* 219: 782, 1938.

10 PETERSBORO

A COMPARISON OF SURFACE TENSION MEASUREMENTS AND HEMOLYTIC ACTIVITY OF GUINEA PIG COMPLEMENT*

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EXPLANATIONS of immunity reactions have frequently been based upon the colloidal nature of sera. The inactivation of complement by heat or exposure to ultraviolet light has been reported to cause an alteration of colloidal state measurable by a lowering of surface tension.^{1, 4} Injections of experimental animals with foreign proteins, bacteria, and gum shellac^{5, 6, 10} have been reported to decrease surface tension, probably through some change in physicochemical equilibrium within the serum, and probably not due to the presence of antibodies. These variations have usually been recorded as "time drops," the difference between initial or dynamic surface tension and static surface tension measured after a two-hour period when all surface active substances are adsorbed in the surface layer. Since a variation in complement potency might be correlated with a change in surface tension, the time drops of a number of sera were determined during a study of guinea pig complement.

Blood serum loses its hemolytic activity when heated to 56° C. According to Zinsser,¹ several investigators reported that during heating there was an aggregation of particles which lowered the surface tension. Upon standing, the complement spontaneously reactivated itself, accompanied by a gradual restoration of surface tension. The gradual deterioration of unheated complement on standing was compared to the slow settling out of colloidal suspensions. Shaking was reported to cause inactivation of complement, probably by coagulation, but whether this is accompanied by a drop in surface tension was not investigated.

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Schmidt² reported that the surface tension of a serum did not permit any inference to be drawn as to its complement activity. Segale³ verified a lowering of surface tension upon heating or aging serum. Ultramicroscopic examination revealed aggregates of particles, whose removal restored the surface tension. The addition of these to fresh sera lowered surface tension but did not change the complement activity. Neither Schmidt nor Segale used the du Noüy tensiometer. Brooks,⁴ using a Traube stalagmometer, found a slight decrease in surface tension of complement after inactivation by ultraviolet light, but believed this was like that found after thermo-inactivation and not the cause of the change in hemolytic power.

Much work on surface tension of serum was done by du Noüy who, through immunization experiments, showed that sera of immune animals gave a greater time drop than normal animals.^{5, 6} Sera of immunized animals reached a maximum time drop in about thirteen days, then decreased and finally disappeared in about thirty days, with further immunization of the same or different kind causing no more change. Thus the injection of an antigen resulted in a physicochemical disturbance measurable by an increased time drop, possibly independent of antibody formation.

Yagle⁷ found little value in surface tension measurements of normal and syphilitic sera and concluded that reagins were not more surface active than other substances in normal sera.

Ramsdell⁸ used a du Noüy tensiometer for surface tension measurements to obtain evidence of a denaturing effect of precipitin reactions upon either antigen or immune serum, and obtained negative results. He⁹ showed an increased time drop with serum from sensitized guinea pigs and concluded that antibody formation could not be involved since the sensitized state remained after the surface tension of the serum had returned to normal.

Following the intravenous injection of gum shellac, Hayman¹⁰ detected an increased time drop and believed this indicated changes in the physicochemical state of plasma.

EXPERIMENTAL

Guinea pigs were lightly etherized and 2 c.c. of heart's blood was aseptically withdrawn for complement. This was placed in a sterile agglutination tube, slanted, allowed to stand at room temperature for one hour, and then placed in a refrigerator (7° to 9° C.) for twenty-four hours before the titer was determined. Each sample was centrifuged to obtain clear serum. The hemolysin, which had a titer of 1:6,000, was obtained from Difco Laboratories.

Corpuscles were obtained from a ewe, blood from the jugular vein being aseptically aspirated into a 30 c.c. syringe, immediately expelled into a sterile flask containing glass beads, shaken for about ten minute to defibrinate the blood, and refrigerated. The defibrinated corpuscles were washed the same day, three times or more, packed by centrifuging for twenty minutes, and made up to a 50 per cent suspension, refrigerated, and used within two days. The corpuscles were made up to a 2 per cent suspension in saline for use in the tests. Throughout the work all dilutions were made with day-old saline only¹¹ prepared from 8.5 Gm. of C. P. sodium chloride and 0.1 Gm. of magnesium chloride, made up to

1,000 c.c. with cold tap water, and autoclaved for thirty minutes at 15 pounds pressure.

Complement Titration.—The complement was titrated by determining the least amount necessary to bring about complete hemolysis of 0.5 c.c. of a 2 per cent suspension of sheep cells in the presence of two units of hemolysin contained in 0.5 c.c. The complement was diluted 1:10 and varying amounts were used, ranging from 0.06 to 0.30 c.c., with 0.02 c.c. intervals. The total volume was made up to 3 c.c. with saline; saline, hemolysin, and complement controls being set up for each titration and titers recorded after a one-hour incubation in a water bath at 37° C.

Surface Tension Determination.—Sera for surface tension measurements were diluted with 0.9 per cent saline, made with C.P. sodium chloride in distilled water. All glassware used in measuring surface tension was soaked for several days in cleaning solution, thoroughly rinsed in distilled water, and dried before use. Determinations were made with a du Noüy tensiometer,¹² using 1.5 c.c. of the diluted serum placed on a watch glass 7.5 cm. in diameter. A measurement was made in about twenty seconds, and after the initial reading the watch glass was set aside for two hours before the second or static reading was made. Subtracting the first reading from the second gave the time drop. The samples of sera were first titrated and the surface tension was then determined after an interval of not more than six hours.

Preliminary surface tension measurements using undiluted serum and dilutions of 1:10, 1:100, 1:1,000, 1:5,000, 1:10,000, and 1:100,000 showed the greatest time drop was at a dilution of 1:10,000, thus agreeing with du Noüy's results on normal and immune sera. Accordingly, all time drops are reported on dilutions of 1:10,000 and at the end of a two-hour period since equilibrium is reached in approximately this time. All measurements were made in a small room free from jarring and air currents, and at a temperature between 21° and 23° C. Between 10 and 15 samples were tested at one time.

Samples.—Samples of sera were obtained from pregnant female, nonpregnant female, and male guinea pigs. Only normal guinea pigs were bled, and their weights varied between 250 and 900 Gm. Feed was removed for eighteen hours before bleeding to avoid chylous sera and only clear sera were used. Samples 1 to 27 were sera of males, the next 18 were taken from females that were not obviously pregnant, and the last 7 samples of sera were from pregnant animals.

The surface tension measurements and hemolytic titers of 52 guinea pig sera are presented in Table I. The males, pregnant and nonpregnant females are listed separately, and the sera are grouped according to titer. Only the time drops and the titers are tabulated.

The differences in time drops of sera from individual males placed in the same group according to titer, and the fact that the differences in time drops between the most potent and least potent sera were no greater than those found in the same titer group, show that the surface tension measurements were without relation to hemolytic activity. Likewise no definite relationship between titers and surface tension measurements of either pregnant or nonpregnant female sera could be established.

TABLE I

COMPARISON OF SURFACE TENSION AND TITER OF GUINEA PIG COMPLEMENT

MALES			FEMALES (NONPREGNANT)			FEMALES (PREGNANT)		
SERUM	TITER	TIME DROP (DYNES)	SERUM	TITER	TIME DROP (DYNES)	SERUM	TITER	TIME DROP (DYNES)
16	0.06*	12.4*	39	0.10*	7.7*	52	0.18*	14.7*
23	0.06	12.9	37	0.10	8.0			
			36	0.10	13.4	50	0.20	12.6
20	0.08	7.4						
7	0.08	8.0	31	0.12	10.0	49	0.22	9.6
1	0.08	9.7				51	0.22	12.0
19	0.08	10.8	28	0.14	12.3			
26	0.08	11.0	44	0.14	16.8	48	0.26	12.5
24	0.08	11.2						
			29	0.16	12.7	47	0.30	9.0
8	0.10	6.3				46	0.30+	17.0
1	0.10	7.0	38	0.18	10.4			
2	0.10	7.1	32	0.18	12.5			
15	0.10	8.4						
18	0.10	8.5	33	0.20	7.8			
10	0.10	9.3	45	0.20	8.6			
21	0.10	9.5	35	0.20	9.6			
25	0.10	12.1	30	0.20	15.1			
17	0.10	13.2						
22	0.10	14.0	42	0.22	15.9			
27	0.10	14.3	43	0.22	18.6			
9	0.12	5.3	40	0.24	12.1			
5	0.12	8.2	41	0.24	12.5			
3	0.12	8.4						
4	0.12	9.0	34	0.26	10.0			
6	0.14	8.8						
14	0.14	11.4						
12	0.16	11.1						
13	0.18	8.1						

*Surface tension measurements were made with sera dilutions of 1:10,000; titrations with 1:10 dilutions.

Time drops of the same value were obtained in the three groups of animals irrespective of titer. If all time drops and titers are compared regardless of their source, such wide variations are apparent that no definite correlation can be found. The two female groups tended to show greater time drops, the meaning of which is obscure. Pregnancy might interfere with the surface tension of sera, but this apparently did not account for the nonpregnant group.

SUMMARY

The hemolytic titers of 27 male guinea pigs ranged from 0.06 to 0.18 c.e., 17 being 0.08 or 0.10 c.e. On the whole, the titers of the 18 nonpregnant females were lower than those of the males, possibly because these females were selected from smaller animals. The highest titer obtained from these females was 0.10 c.e., and the lowest was 0.26 c.e., the 18 samples so distributed between these titers that no one titer group predominated.

Pregnancy definitely lowered the titer of 7 guinea pig complements examined. However, the surface tension as measured by time drops in some instances were no greater than those recorded for nonpregnant females or males with materially higher titers. Nevertheless a larger percentage of sera from females had higher time drops than those from males.

A larger percentage of sera from males showed time drops of less than 10 dynes than was recorded for either pregnant or nonpregnant females. Sixty

per cent of the males showed a time drop of less than 10 dynes, while approximately 27 and 30 per cent of nonpregnant and pregnant females, respectively, had time drops of less than 10 dynes.

It must be concluded that the surface tension, expressed as time drop, of 52 samples of guinea pig complement measured with a du Noüy tensiometer, showed no correlation with hemolytic activity.

REFERENCES

1. Zinsser, H.: *Resistance to Infectious Diseases*, ed. 4, New York, 1931, The Macmillan Company, pp. 226-228.
2. Schmidt, H.: Complement Action in Regard to Surface Tension, *J. Hyg.* 13: 314, 1913-1914.
3. Segale, M.: Relation of Complement of Serum to Surface Tension of Serums, *Inst. Pathol. Gen. Univ. Genoa, Patol.* 1: 709; *Through Ztschr. f. Immunitätsforsch. u. Exper. Therap. Ref.* 4: 385. Reviewed in *Chem. Abstr.* 5: 3700, 1911.
4. Brooks, S. C.: The Mechanism of Complement Action, *J. Gen. Physiol.* 3: 185, 1920.
5. du Noüy, P. L.: The Relation Between Time Drop and Serum Antibodies, *J. Exper. Med.* 37: 659, 1923.
6. du Nody, P. L.: On Certain Physicochemical Changes in Serum as a Result of Immunization, *J. Exper. Med.* 41: 779, 1925.
7. Yagle, E. M.: Surface Tension of Blood Serum in Syphilis, *J. Immunol.* 16: 17, 1929.
8. Ramsdell, S. G.: Surface Tension of Sera as Affected by the Precipitin Reaction, *J. Exper. Med.* 48: 615, 1928.
9. Ramsdell, S. G.: Surface Tension of Serum of the Sensitized Guinea Pig, *J. Exper. Med.* 47: 987, 1928.
10. Hayman, J. M.: The Effect of Gum Shellac Solution on the Surface Tension of Rabbit Serum, *J. Exper. Med.* 45: 771, 1927.
11. Faber, J. E., and Black, L. A.: The Influence of Physiologic Salines in Complement Fixation Reactions, *J. Lab. & Clin. Med.* 21: 1069, 1936.
12. du Noüy, P. L.: An Apparatus for Measuring Surface Tension, *J. Gen. Physiol.* 1: 521, 1919.

THE EFFECT OF CARBON TETRACHLORIDE AND THYROXIN ON THE DEVELOPMENT OF SENSITIVITY TO EGG ALBUMEN AND RAGWEED POLLEN EXTRACTS IN THE GUINEA PIG*

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THE present study was undertaken to determine whether acute anaphylactic shock in the guinea pig produced with extracts of low ragweed pollen might be achieved more certainly and consistently than had been reported by other workers.¹⁻⁸ Eagle and co-workers⁸ have demonstrated that by employing adequate doses of extracts of low ragweed pollen approximately 50 per cent of guinea pigs might be sensitized to such a degree that fatal anaphylactic shock could be induced. We decided to attempt sensitization of our animals at a time when they were undergoing profound metabolic disturbances. Under these conditions rapid destruction of the antigen or overproduction of antibodies, factors conceivably having an adverse effect on the development of a high degree of sensitivity, might be altered. Carbon tetrachloride was chosen as one agent because it injures the liver, an organ containing an important part of the reticulo-endothelial system. Thyroxin was used in another experiment

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because of its effect on the metabolism of the organism as a whole. Because attempts to affect the development of sensitivity by producing metabolic disturbance in the test animal at the time sensitizing doses of antigen were administered have been almost exclusively limited to extirpation of organs (Hill and Martin⁹), it seems worth while to report these studies.

There has been to our knowledge only one study on the possible relationship of liver necrosis to experimental anaphylaxis. Paul and Roth,¹⁰ using phosphorus as the toxic agent in guinea pigs, produced results which indicated that acute phosphorus poisoning interferes with anaphylactic shock and that subacute phosphorus poisoning present at the time of sensitization prevents the acquisition of sensitivity to horse serum in 50 per cent of guinea pigs. One other study is of interest in this connection. Mills and Dragstedt,¹¹ in an effort to blockade the reticulo-endothelial system of the liver, obstructed the common bile duct and produced a bilirubinemia in eight dogs. This treatment did not prevent anaphylactic shock. In all but one instance the operation was done at least one week after the sensitizing dose of horse serum had been given. In one dog the common bile duct was obstructed two days prior to sensitization. This animal was successfully shocked twelve days later. No statement was made as to the presence or absence of necrosis in the livers of these dogs. These workers have also demonstrated that blockading the reticulo-endothelial system with India ink and saccharated iron oxide will not prevent anaphylactic shock in dogs.¹² They discussed the conflicting reports of results of blockade in small animals, as summarized by Hill and Martin. Citing some experimental work of their own, they concluded that it is important to administer the shocking dose of horse serum intravenously. The prior injection of dyes seemed to affect absorption of the antigen from the peritoneal cavity. It seemed worth while, however, to investigate the possibility that nonspecific disturbances of liver function might promote sensitization. We, therefore, administered the sensitizing doses of antigen at the time of the maximal effect of nonfatal carbon tetrachloride poisoning.

Képinow and Lanzenberg,^{13, 14} studying the effect of thyroidectomy on anaphylaxis in guinea pigs, concluded that if thyroidectomy was performed prior to sensitization procedures no sensitization resulted, but that thyroidectomy done twenty days after sensitization and seven days prior to shocking did not inhibit anaphylaxis. Moreover, thyroidectomized animals given thyroid substance during the period of sensitization died of anaphylactic shock. Passive transfer studies confirmed their opinion that normal thyroid function was essential to the production of anaphylactic antibodies, but was not essential to anaphylactic shock.^{14, 15} Appelmans¹⁶ reported a few experiments which do not confirm this view. Pfeiffer,¹⁷ however, was able to substantiate the results of Képinow and Lanzenberg. It seemed possible, therefore, that giving sensitizing doses of antigen at a time when the animal was undergoing the profound metabolic disturbances induced by a single massive dose of thyroxin might result in the sensitization of a greater proportion of animals.

METHODS

Phelps and Hu¹⁸ produced definite necrosis of the liver in guinea pigs by the oral administration of 0.66 c.c. of carbon tetrachloride per kilogram of body

weight. We determined, in a series of preliminary studies, that in guinea pigs weighing over 400 Gm., 1.0 c.c. of carbon tetrachloride per kilogram of body weight would regularly produce a definite necrosis of the liver demonstrable on the fifth and seventh days after subcutaneous inoculation. This dosage proved to be too high, for in one series most of the pigs died a day or two after receiving the initial sensitizing doses of ragweed pollen extract, given at the time of, or just prior to, the expected maximum liver damage. In Experiment II a dose of 0.8 c.c. of carbon tetrachloride per kilogram of body weight was used. For small animals, of 300 to 400 Gm. body weight, 0.6 c.c. per kilogram was injected.

The carbon tetrachloride was administered subcutaneously as a 50 per cent solution in olive oil.

The thyroxin solution was prepared freshly a few minutes prior to subcutaneous inoculation by dissolving 10 mg. of Squibb's crystalline thyroxin in one drop of 1 N sodium hydroxide and then making it up to 10 c.c. with distilled water. The egg albumen was a 1:10 dilution of fresh egg albumen in plain physiologic salt solution.

Pollen extracts were prepared by adding the desired amount of low ragweed pollen obtained from Knapp and Knapp to an amount of physiologic sodium chloride solution sufficient to give the desired concentration. This mixture was kept at room temperature for two hours during which time it was agitated repeatedly. It was then placed in a refrigerator. The following day the mixture was filtered through a sterile Seitz filter. The extract was then ready for use. Extracts used for sensitizing were kept in the refrigerator for as long as five days after preparation. Extracts used for shocking were prepared freshly for each day's experiments. Extracts of a 1:20 concentration contained approximately 0.72 mg. of nitrogen per cubic centimeter, and the 1:10 extracts, 1.5 mg. of nitrogen per cubic centimeter. All sensitizing doses were administered intraperitoneally. The shocking doses were given into the jugular vein of the unanesthetized animal. All the guinea pigs used in these experiments were kept on a diet of ground "Alfa Rabbit Flakes" with daily greens in the form of lettuce, celery tops, and occasionally cabbage. The animals were weighed at the time of their first inoculations. With the exceptions noted later they were in good health for the duration of the experiments.

EXPERIMENTAL

Experiment I. Eight guinea pigs, weighing from 450 to 680 Gm., were given 1 c.c. of carbon tetrachloride per kilogram of body weight. On the third and sixth days following, 0.5 c.c. of a 1:10 egg albumen solution was injected intraperitoneally. On the twenty-first day after the last injection 0.5 c.c. of a 1:10 egg albumen solution was given intravenously. Six animals died within four minutes of the injection in typical acute anaphylactic shock. Autopsy confirmed the diagnosis. Treatment with carbon tetrachloride did not interfere with the development of sensitivity to egg albumen.

Experiment II. (Table I) We next treated 6 male and 6 female guinea pigs with 0.8 c.c. of carbon tetrachloride per kilogram of body weight. Sensitizing doses of a 1:20 extract of low ragweed pollen were given on the third, fifth, and

seventh days following administration of the carbon tetrachloride. Simultaneously a similar group of 12 control animals was given sensitizing doses. One animal (206) in the first group died of hemorrhage, the result of administration of the first dose of pollen extract on the third day following injection of carbon tetrachloride. Microscopic study of the liver from the animal showed a large accumulation of fat and considerable necrosis about the centers of the liver lobules. The shock doses of fresh 1:10 extract of low ragweed pollen were

TABLE I
(Experiment II)

GUINEA PIG NO.	SEX	WT. IN GRAMS	3/3/39 50% CCl ₄ IN OLIVE OIL	1:20 EXTRACT S.R.W.†			RESPONSE 3/29-30/39 0.5 C.C. 1:10 EX- TRACT OF SMALL RAGWEED INTRA- VENOUSLY
				3/6/39	3/7/39	3/9/39	
207	F	450	0.75 c.c. s.c.*	1 c.c. i.p.†	1 c.c. i.p.	1 c.c. i.p.	None
211	F	500	0.8 c.c. s.c.	1 c.c. i.p.	1 c.c. i.p.	1 c.c. i.p.	Death in 1 minute
206	F	510	0.8 c.c. s.c.	Died from hemorrhage			
205	F	470	0.77 c.c. s.c.	1 c.c. i.p.	1 c.c. i.p.	1 c.c. i.p.	None
210	F	420	0.7 c.c. s.c.	1 c.c. i.p.	1 c.c. i.p.	1 c.c. i.p.	None
222	F	470	0.77 c.c. s.c.	1 c.c. i.p.	1 c.c. i.p.	1 c.c. i.p.	Death in 3 minutes
252	M	440	0.7 c.c. s.c.	1 c.c. i.p.	1 c.c. i.p.	1 c.c. i.p.	None
272	M	460	0.75 c.c. s.c.	1 c.c. i.p.	1 c.c. i.p.	1 c.c. i.p.	Died 3/21/39—peri- tonitis
251	M	440	0.7 c.c. s.c.	1 c.c. i.p.	1 c.c. i.p.	1 c.c. i.p.	None
214	M	450	0.75 c.c. s.c.	1 c.c. i.p.	1 c.c. i.p.	1 c.c. i.p.	None
267	M	400	0.65 c.c. s.c.	1 c.c. i.p.	1 c.c. i.p.	1 c.c. i.p.	None
263	M	460	0.75 c.c. s.c.	1 c.c. i.p.	1 c.c. i.p.	1 c.c. i.p.	Died 3/21/39—peri- tonitis
203	F	460		1 c.c. i.p.	1 c.c. i.p.	1 c.c. i.p.	None
220	F	490		1 c.c. i.p.	1 c.c. i.p.	1 c.c. i.p.	Death in 5 minutes
221	F	350		1 c.c. i.p.	1 c.c. i.p.	1 c.c. i.p.	Death in 3½ minutes
213	F	530		1 c.c. i.p.	1 c.c. i.p.	1 c.c. i.p.	Death in 3¼ minutes
223	F	500		1 c.c. i.p.	1 c.c. i.p.	1 c.c. i.p.	None
218	F	460		1 c.c. i.p.	1 c.c. i.p.	1 c.c. i.p.	Death in 4½ minutes
274	M	440		1 c.c. i.p.	1 c.c. i.p.	1 c.c. i.p.	None
269	M	430		1 c.c. i.p.	1 c.c. i.p.	1 c.c. i.p.	Death in 8¼ minutes
254	M	440		1 c.c. i.p.	1 c.c. i.p.	1 c.c. i.p.	None
259	M	450		1 c.c. i.p.	1 c.c. i.p.	1 c.c. i.p.	None
270	M	530		1 c.c. i.p.	1 c.c. i.p.	1 c.c. i.p.	Death in 5 minutes
273	M	440		1 c.c. i.p.	1 c.c. i.p.	1 c.c. i.p.	None
262	M	460	0.7 c.c. s.c.				None
225	M	390					None
255	M	430					None

*Subcutaneously.

†Intraperitoneally.

‡Low ragweed.

TABLE II
(Experiment III)

GUINEA PIG NO.	SEX	WT. IN GRAMS	2/14/39		2/15/39	2/16/39	RESPONSE 3/8/39 0.5 C.C. 1:10 EX- TRACT OF SMALL RAGWEED INTRA- VENOUSLY
			2:15 P.M. MG. THYRONIN	4:00 P.M. C.C. 1:20 EXTRACT S.R.W.	2:30 P.M. 1:20 EX- TRACT S.R.W.	2:00 P.M. 1:20 EX- TRACT S.R.W.	
287	M	350	0.35	1 c.c. i.p.	1 c.c. i.p.	1 c.c. i.p.	Death in 2 minutes
289	M	370	0.35	1 c.c. i.p.	1 c.c. i.p.	1 c.c. i.p.	Death in 4 minutes
298	M	410	0.35	1 c.c. i.p.	1 c.c. i.p.	1 c.c. i.p.	Death in 2 minutes
217	M	350	0.35	1 c.c. i.p.	1 c.c. i.p.	1 c.c. i.p.	None
215	M	405	0.35	1 c.c. i.p.	1 c.c. i.p.	1 c.c. i.p.	None
209	M	345	0.35	1 c.c. i.p.	1 c.c. i.p.	1 c.c. i.p.	Slight symptoms

given intravenously on the twentieth and twenty-first days following the last sensitizing dose. The extracts were tested on normal animals for possible toxicity, but the results were negative. As will be seen from Table I, 50 per cent of the animals not treated with carbon tetrachloride were rendered sufficiently sensitive to die in acute anaphylactic shock. Only 2 of the 9 animals treated with carbon tetrachloride, and surviving until the shock date, died from the intravenous injection. Apparently carbon tetrachloride as administered did not favor the development of sensitivity to extracts of ragweed pollen.

Experiment III. (Table II.) Six guinea pigs, weighing 350 to 450 Gm. each, were given 35 mg. of thyroxin subcutaneously. Two, twenty-four, and forty-eight hours later, 1 c.c. of a 1:20 extract of low ragweed pollen was given intraperitoneally. Three weeks later 3 of the 6 animals died in typical acute anaphylactic shock following intravenous administration of 0.5 c.c. of 1:10 pollen extract. These results are similar to those obtained in the control group in Experiment II.

SUMMARY

1. Treatment of guinea pigs with carbon tetrachloride, and the administration of sensitizing doses of egg albumen at the time of maximal anatomic change, did not prevent the acquisition of sensitivity to egg albumen in 6 of 8 animals.

2. Two of 9 guinea pigs given sensitizing doses of an extract of low ragweed pollen at a time when they were suffering from carbon tetrachloride poisoning died from typical anaphylactic shock after intravenous administration of pollen extract twenty-one days later.

3. Fatal anaphylactic shock was produced with simple saline extracts of low ragweed pollen in 6 of 12 normal guinea pigs.

4. Three of 6 guinea pigs receiving sensitizing doses of extracts of low ragweed pollen at a time of maximal effect from a single massive dose of thyroxin died in typical anaphylactic shock after receiving the pollen extract intravenously.

CONCLUSION

1. Carbon tetrachloride as employed in these experiments does not appear to inhibit the sensitization of guinea pigs to egg albumen. On the other hand, it does not favor the development of sensitivity to extracts of low ragweed pollen.

2. Thyroxin, as used in these experiments, does not appear to affect the development of sensitivity to extracts of low ragweed pollen.

3. One-half cubic centimeter of a simple saline extract of low ragweed pollen, containing approximately 0.75 mg. of nitrogen, is capable of producing typical fatal anaphylactic shock in sensitized guinea pigs.

REFERENCES

1. Dunbar, W. P.: The Present State of Our Knowledge of Hay Fever, *J. Hyg.* 13: 105, 1913.
2. Cooke, R. A., Flood, E. P., and Coca, A. F.: Hay Fever, *J. Immunol.* 2: 219, 1917.
3. Harrison, W. T., and Armstrong, C.: Some Experiments on the Antigenic Principles of Ragweed Pollen Extracts, *U. S. Pub. Health Rep.* 39: 1261, 1924.
4. Walzer, M., and Grove, Ella F.: Studies in Specific Hypersensitiveness, XVI. On Antigens, *J. Immunol.* 10: 483, 1925.

5. Huber, H. L., and Koessler, K. K.: The Antigenic Property of Pollens, Arch. Int. Med. 36: 751, 1925.
6. Caulfeild, A., Cohen, C., and Eadie, G.: The Antigenic Properties of Pollen Fractions, J. Immunol. 12: 153, 1926.
7. Bernstein, C.: Studies on Anaphylaxis With Pollen, J. Exper. Med. 61: 149, 1935.
8. Eagle, Harry, Arbesman, C. E., and Winkenwerder, W. L.: The Production in Experimental Animals of Antibodies to Short-Ragweed Pollen, J. Immunol. 36: 425, 1939.
9. Hill, Justin, and Martin, L.: A Review of Experimental Studies on Non-specific Inhibition of Anaphylactic Shock, Medicine 11: 141, 1932.
10. Paul, B., and Roth, E.: Leber Funktion und anaphylaktischer Shock, Ztschr. f. d. ges. exper. Med. 94: 554, 1934.
11. Mills, M. A., and Dragstedt, C. A.: Common Bile Duct Obstruction and Anaphylaxis, Proc. Soc. Exper. Biol. & Med. 34: 469, 1936.
12. Mills, M. A., and Dragstedt, C. A.: The Reticulo-endothelial System and Anaphylaxis in the Dog, J. Immunol. 31: 1, 1936.
13. Lanzenberg, A., and Képinow, L.: Glande thyroïde et Anaphylaxie, Compt. rend. Soc. de biol. 86: 204, 1922.
14. Képinow, L., and Lanzenberg, A.: Glande thyroïde et Anaphylaxie, Compt. rend. Soc. de biol. 86: 206, 906, 1922.
15. Képinow, L., and Lanzenberg, A.: Glande thyroïde et Anaphylaxie, Compt. rend. Soc. de biol. 87: 409, 494, 1922.
16. Appelmans, R.: Glande thyroïde et Anaphylaxie, Compt. rend. Soc. de biol. 87: 1242, 1922.
17. Pfeiffer, H.: Über den Einfluss des Schilddrüsenverlustes auf die Wärmeregulation des Meerschweinchens, Arch. f. exper. Path. u. Pharmacol. 98: 253, 1923.
18. Phelps, B. M., and Hu, C. H.: Carbon Tetrachloride Poisoning, J. A. M. A. 82: 1254, 1924.

950 EAST 59TH STREET

A CASE OF *STRONGYLOIDES STERCORALIS* INFESTATION*

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THE incidence of *Strongyloides stercoralis* infestation is rare in the northern parts of the United States. Ginsburg reported a case in Pennsylvania, and Cadham reported one in Winnipeg, Canada. The parasite is more common in the subtropical and tropical countries and is quite common in the southern parts of the United States. Faust estimated that 6 per cent of the clinic population is infested in New Orleans.

The life cycle of this parasite belonging to the nematode group is quite similar to the *Ankylostoma duodenale*. The female larvae live in the duodenum and jejunum. From the eggs the rhabditoid larvae develop. These in turn may either develop into the strongyloid larvae (infesting forms) or into male or female rhabditoid larvae. These copulate and the eggs burst into the first rhabditoid larvae, and the life cycle starts all over again. The infestation occurs either through the skin by the strongyloid larvae or through the oral cavity. From the skin the larvae get into the blood stream, lungs, trachea, esophagus, and the small intestine.

The case presented here is of interest because it occurred in New York State where the incidence of *Strongyloides* infestation is rare and because its clinical aspects are unusual.

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CASE HISTORY

M. M., a 45-year-old woman, was born in Italy and migrated to this country twenty years ago with her husband. Since that time she has lived in East Rochester. She has three children.

She gave a long previous history of uncertain abdominal pain; she vomited at times and was sometimes jaundiced, the latter being only her own observation. For these uncertain complaints she was hospitalized several times in different hospitals of this city and underwent three abdominal operations: in 1933 cholecystectomy and appendectomy, in 1935 and again in 1938 exploration for adhesions. All these operations showed negative findings. One blood count, however, done in 1933 showed an eosinophilia of 10 per cent.

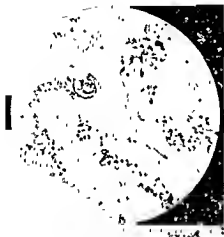


Fig. 1.



Fig. 2.

Fig. 1.—Rhabditoid larva of *Strongyloides stercoralis*. Low power.

Fig. 2.—Oral end of the parasite. a, First cylindrical dilatation. b, Second bulbous dilatation with chitinous Y. High power.



Fig. 3.

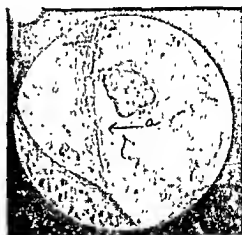


Fig. 4.

Fig. 3.—Body part of the parasite with primitive genital organ (a). High power.

Fig. 4.—Tail end with anal opening (a). High power.

On admission to St. Mary's Hospital on Jan. 30, 1940, she complained of colicky pain, starting in the right upper quadrant and sweeping across the abdomen to the left side. The pain was also felt in the back. About one hour after the onset she vomited some bloody material.

The physical examination was essentially negative. Pressure in the left lower quadrant produced pain in the epigastrium.

The red blood cell count and hemoglobin showed a slight hypochromic anemia. The white blood cell count ranged between 6,000 and 8,000, with a marked eosinophilia of 25 to 29 per cent eosinophile cells. The urine was negative. Routine blood chemistry and the Wassermann reaction were negative.

The first stool examination did not show any parasites, but a large number of Charcot-Leyden crystals. The second stool examination after administration of a saline laxative showed numerous vividly motile nematodes. These parasites were from 200 to 300 microns long and showed marked wriggling motions (Fig. 1). Under high power the oral end showed a short mouth which opens into the esophagus, showing first a cylindrical then a bulbous dilatation followed by the intestine (Fig. 2). In the body on both sides of the intestine ellipsoid structures could be seen constituting the primitive genital organs (Fig. 3). The tail end was pointed and on the base of the pointed tail a cuticular elevation was found which formed the anal opening (Fig. 4). On the basis of these characteristics the parasite could be classified as the first rhabditoid larva of *Strongyloides stercoralis*.

I kept the stool specimens for twenty-four hours in the incubator (37° C.) and by examining the stool specimen I found that the larvae developed into the strongyloid forms. These larvae were much longer than the previous ones and showed well-coordinated boring motions (Fig. 5). After these findings repeated stool examinations were performed and larvae were always found to be present. Gastric analysis and sputum examinations were also done, but these did not reveal the presence of parasites. A skin test with trichinella antigen was positive in 1:1,000 dilution (group reaction).

The treatment consisted of duodenal lavage and gentian violet tablets, 1 grain three times daily. The patient was not very cooperative; she left the hospital and repeated stool examinations still show the presence of the parasite.



Fig. 5.—Strongyloid form of the parasite. Low power.

The stools of the husband and the children were also examined, and the parasite was found to be present in the husband's stool. The blood count of the husband showed eosinophilia of 10 per cent, but otherwise he did not show any symptoms which could be related to the presence of the parasite.

DISCUSSION

Both individuals undoubtedly acquired the parasite in Italy, where the presence of the parasite is known, and they harbored it in their intestines all the time. In the husband it seemingly did not cause any symptoms, except the slight eosinophilia. It is somewhat more difficult to relate the parasite as an etiologic factor to the clinical symptoms in the woman. We do not have a history of an initial itch or eczema at the time of infestation. This condition, however, may be easily overlooked by a woman doing heavy housework. On the other hand, the infestation may have occurred by mouth.

According to the literature, the *Strongyloides stercoralis* is well accepted as a causative agent of diarrhea. The first cases reported were the so-called "Cochin-China" diarrhea in soldiers by Normand. There are also several fatal cases of Strongyloidosis reported in which post-mortem examinations were

performed. Ophuls found the larvae in the intestinal wall and in the mesenteric lymph nodes. Gage found them in the lymph spaces of the duodenal wall. Proes found them in his case in the pleural effusion, pericardial fluid, and in the blood.

Both types of larvae have very intensive motion capacities, and there is no reason why they could not get into the channels connected with the gastrointestinal tract. It is very easily possible that the parasite might get actively or passively into the lumen of the appendix and even without causing a true "appendicitis," through the irritative action of their presence cause appendicular colic which might be easily interpreted as an acute appendicitis.

In the same manner they might migrate into the gall ducts or the gall bladder and cause symptoms of a gall-bladder disease. Nisbet reported a case in which the worm caused obstructive jaundice. There is no reason why they could not get in the same manner into the pancreatic duct and add to the uncertainty of the abdominal symptoms.

The negative findings of the removed surgical specimens and the negative findings at the explorations, together with the preservation of the symptoms after the operations, are enough proof that the patient did not have a true cholecystitis or appendicitis or any other condition which would have resulted in pathologic changes of the abdominal organs. In my opinion the uncertain abdominal symptoms were caused by the presence of the parasite. As far as her present symptoms are concerned, the question is undecided as to whether they are caused by the presence of the parasite or whether they are only neurotic sequelae after her three previous abdominal operations. It is a known fact that individuals harboring parasites develop severe neuroses.

SUMMARY

A case of an Italian immigrant woman is presented in which the stool examinations revealed rhabditoid larvae of *Strongyloides stercoralis*.

It may be assumed that the parasite caused uncertain abdominal symptoms for which the gall bladder and the appendix were removed. Later two laparotomies were performed for the persistence of symptoms, which were thought to be due to postoperative adhesions. All operations resulted in negative findings.

REFERENCES

- Calham, F. T.: Infestation With *Strongyloides stercoralis*, Associated With Severe Symptoms, *Canad. M. A. J.* 29: 18, 1933.
 Faust, E. C.: The Symptomatology, Diagnosis and Treatment of *Strongyloides* Infection, *J. A. M. A.* 98: 2276, 1932.
 Gage, I.: A Case of *Strongyloides Intestinalis* With Larvae in the Sputum, *Arch. Int. Med.* 7: 561, 1911.
 Ginsburg, L.: *Strongyloides Intestinalis* Infestation, *J. A. M. A.* 75: 1137, 1920.
 Normand, A.: Sur la maladie dite diarrhee de Cochinchine, *Compt. rend. Acad. d. sc.* 5: 316, 1876.
 Nisbet, D. H.: Obstructive Jaundice Due to *Strongyloid* Larvae, *South. Med. & Surg.* 94: 785, 1932.
 Ophuls, W.: A Fatal Case of *Strongyloidosis* in Man With Autopsy, *Arch. Path.* 8: 1, 1929.
 Proes, H. E.: The Biology of *Strongyloides stercoralis* Thesis, Bahia, 1930, *Arch. Path.* 13: 168, 1932.
 Simpson, V. E.: *Strongyloidiasis*, *J. A. M. A.* 112: 828, 1939.

RELATION OF THE MACRONUCLEOLUS AND NUCLEONUCLEOLAR RATIO OF CANCER TO HISTOLOGIC GRADING*

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THE pathologic diagnosis of cancer has interested many students, and the practical value of such a diagnosis depends on the early recognition of the condition. For this reason, the attention of pathologists during the last few years has been focused on the cell as the unit of cancerous tissue. Because there has been no reliable chemical or serologic test for cancer, the proper treatment has been based on an early clinical suspicion of the disease, its early pathologic recognition, and the determination of the degree of malignancy. Since Virchow established cellular pathology, pathologists have tried to find in the cells of the cancerous tissue some signs on which they could rely for diagnosis, particularly in the early stages of the disease.

After reading the papers of MacCarty dealing with the diagnosis of cancer on the basis of the macronucleolus of its cellular constituents, and the papers of Broders on the grading of malignancy according to the percentage of cell differentiation, I began to study many specimens of fresh cancerous tissue. The work of MacCarty and the work of Broders not only are of great practical value but also are very interesting from a scientific standpoint, and are closely related. The more slides I studied, the more strongly was I convinced that there was really a cancer cell which had a markedly increased nucleolar substance and that in the great majority of cases the amount of that substance varied according to the degree of malignancy. It was on this basis that the present work was done. It attempts to prove that there is a marked increase of nucleolar material in the cancer cell and that the amount of this material is directly proportional to the degree of malignancy.

The presence of one or more large nucleoli in the cells of embryonic and regenerative tissues, and in any normal tissue which is metabolically very active, suggests that the increased size of the nucleolus is a direct consequence of the great cellular activity. As cellular division is one of the greatest expressions of cellular activity, it seems reasonable to admit that any condition in which cellular division is active would afford good opportunity for studying the variation in the size of the nucleolus. It was with this in mind that many months were spent trying to make normal and cancerous tissues grow in vitro. Tissue cultures are, perhaps, the best field in which to study cellular metabolism and cellular division, because cells in vitro grow without body control in a fashion somewhat comparable to the growth of cancer in the body. Unfortunately, because of technical difficulties very few of the cultures grew well, but the few that grew confirmed what has been seen in pictures and descriptions of cultures

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of tissues in vitro—large cells, which had a large nucleus and very large nucleolus.

In 1851 Lebert pointed out that cancer cells possessed one or more large nucleoli, but Vögel, Bennett, and Virchow did much to obscure his work, and the macronucleolus in cancer cells was entirely forgotten until MacCarty in 1923,²² after an exhaustive and interesting research again called attention to the importance of the macronucleolus in the diagnosis of cancer. In a series of papers written from 1923 to 1937, MacCarty²³⁻²⁶ described the "cancer cell" and stressed its characteristics (spheroid or oval form, large nucleus, single or multiple large nucleolus, and low nucleonucleolar ratio), as well as a manner of distinguishing the cells of tertiary cytoplasia from the cells of secondary and primary cytoplasia (MacCarty). However, he repeatedly said that cells of secondary cytoplasia and cancer cells are morphologically very much alike; the only difference is that the former remain in the acini while the latter invade the surrounding stroma. MacCarty and his co-workers continued their study of the nucleonucleolar ratios in malignant and nonmalignant tissues. In 1933 MacCarty, Haumeder, and Berkson made a preliminary report on a series of actual measurements of the nuclear and nucleolar areas and corresponding ratios. They expressed the opinion that the difference between the nucleolar areas in malignant cells and nonmalignant cells is greater than that between the nuclear areas. They said that the nucleonucleolar ratio in malignant cells varies from 1:5 to 1:17, while in nonmalignant cells the ratio varies from 1:13 to 1:45.

In the latter years nuclear and nucleolar areas with the corresponding ratios have been determined in different organs obtained in the laboratory of MacCarty, by Haumeder, McCormack, Naidu, Strohl, Kaump and Fairchild, Kroeze, Cohen, Lofgren, Page, Smith, Campbell, Farthing, and Frugoni. All these authors have definitely confirmed the work of MacCarty,¹⁰ who recently submitted a paper for publication in which he condensed the results of their biometric studies. Similar observations have been made by Stenius, Castren, Saxen, Quensel (1928), Karp (1932), Fidler (1935), and Von Haam and Alexander (1936).

MacCarty recently said: "Microscopic grading has been in existence ever since tissues and tumors have been studied with the aid of microscopes." However, one can say that the real microscopic grading of tumors began in 1920,^{2, 5} when Broders made his first report on the subject. In 1925, after nine years' experience in the grading of epitheliomas, Broders said that a slight modification should be made in his former classification. Thus, tumors should be called "grade 1" when undifferentiation ranges from 0 to 25 per cent, "grade 2" when undifferentiation ranges from 25 to 50 per cent, "grade 3" when undifferentiation ranges from 50 to 75 per cent, and "grade 4" when undifferentiation ranges from 75 to 100 per cent.

In determining the degree of malignancy Broders also considered the number of mitotic figures and the number of cells which have single, large, deeply staining nucleoli (one-eyed cells). Later, in emphasizing the practical value of grading and advising the type of treatment according to grade of malignancy, he said that grade 1 tumors show practically no tendency to metastasize, while grade 4 cancers, metastasize quickly and sooner or later prove fatal. Grade 2

and grade 3 cancers occupy a place between grade 1 and grade 4 as far as metastasis and postoperative results are concerned.

Other methods of grading of tumors, which have been recommended by different authors, are based on cellular differentiation alone or on this and other pathologic and clinical factors. The grading system of Broders, which is the method most often employed in this country, was followed in this work.

MATERIAL AND METHODS

Only human tissues which had been removed surgically were used in this study. The material included 119 malignant and 45 nonmalignant tissues from different organs and tissues, including those obtained from a four-month fetus. Both malignant and nonmalignant tissues were obtained from the thyroid gland, breast, stomach, liver, rectum, sigmoid colon, ovary, endometrium, and cervix uteri. The fetal tissues included the thyroid gland, stomach, liver, and rectum. Only epithelial tissues and tumors of the same origin were studied. The malignant tumors included adenocarcinoma, scirrhus carcinoma, papillary adenocarcinoma, and squamous-cell carcinoma (epithelioma). All the growths were primary except those found in the liver, where metastatic growths also were used. The nonmalignant tissues included normal tissues, subacute and chronic inflammation, hyperplasia, and benign tumors (polyps, adenomas, fibroadenomas, and cysts). As soon as the tissues were obtained from the operating room they were placed in a 10 per cent solution of formalin for fixation. Twenty-four hours later frozen sections from 5 to 10 microns thick were cut and stained with hematoxylin and eosin. The sections were studied histologically and cytologically with low, medium, and high-power objectives, and the malignant tumors were graded according to the classification of Broders.* An attempt was made to obtain approximately the same number of tumors of each grade from each organ. However, this was difficult and even impossible in certain organs. In the thyroid gland, breast, and rectum there were found carcinomas of every grade from 1 to 4. Grade 1 carcinoma very rarely occurs in the stomach and liver. Carcinoma of the ovary most frequently is grade 1 or 2, occasionally it is grade 3, but very rarely grade 4. The most frequent grades of carcinoma found in endometrium are 1 and 2, but grade 3 or 4 carcinoma occasionally may be found in this situation. On the contrary, in the uterine cervix the usual grades encountered are 3 and 4, and grades 1 and 2 only rarely. The cytologic studies were made by using an oil-immersion lens of a Spencer microscope (objective 1.8 mm., N.A. 1.25, tube length 150 mm., and ocular 10 \times). In making the biometric studies a camera lucida was mounted on this microscope, and the contours of the shadows of the nuclei and nucleoli were projected on paper and carefully traced with a sharp pencil. From each section of both malignant and nonmalignant tissue, 20 cells were traced; four cells were chosen in each of five different microscopic fields. These were the cells which had the largest nucleoli. The tracings of the nuclei were measured directly by a planimeter to obtain their areas. Since many nucleoli were small it was necessary to enlarge them in order to obtain measurable tracings. This was done with a camera lucida mounted on a

*The grades of the carcinomas of the breast, stomach, and ovary were kindly checked by Dr. A. C. Broders.

Spencer microscope (objective 48 mm., tube length 250 mm., and ocular 1x). The tracings that were obtained were also measured by a planimeter and the areas were registered. The magnification of this microscope and camera lucida was found to be 42 diameters; therefore, it was necessary to divide the areas of the nucleoli by 42 in order that the magnification might correspond to that employed in the measurement of the nuclei. To obtain the actual areas of the nuclei and nucleoli in square microns, it was necessary to correct the planimeter readings by taking into consideration the magnification of both microscopes, the camera lucida, and the constant of the planimeter.

Under the same conditions as the cells were drawn, a known space of a micromillimeter scale was projected on paper by means of the camera lucida, and the projections were traced and measured. The magnification of the microscope, which was determined in this manner, was found to be 1,800 diameters. The calibration of the planimeter was such that a circular area of 100 sq. cm. represented 2,500 planimeter units. Each area of the planimeter reading was multiplied by a constant that was obtained by using the following formula:

$$\frac{\text{Value for one planimeter unit}}{(\text{Magnification of the microscope})^2} = K.$$

After the actual areas were obtained, it was necessary only to divide the areas of the nuclei by those of the nucleoli in order to obtain the corresponding nucleonucleolar ratio. It is necessary to explain why the quotient

$\frac{\text{average nuclear measurement}}{\text{average nucleolar measurement}}$ does not correspond, except by chance, with the

ratios given. The reason is that the calculation $\frac{\text{average nuclear measurement}}{\text{average nucleolar measurement}}$ was not made. Instead, the nuclear measurement of each cell was divided by the nucleolar measurement of each cell, and these quotients were averaged. The divisions originally were carried out to three decimal places but were contracted later so that it would be necessary to print only one or two decimal places. These contractions caused some lack of correspondence, more apparent than real, between the results of calculations made in the various possible ways.

RESULTS

It would be outside of the purpose of this work to describe the histologic arrangement of the cells in the normal, inflammatory, and neoplastic tissues studied. A description of this arrangement may be found in textbooks and various papers. However, it seems appropriate to consider briefly the variations in size, shape, and number of the nucleoli. Ehrlich recently said, that "the nuclear sizes of normal tissues are by no means constant." The same can be said about the size of the nucleolus. The nucleolus was not easily seen in the cells of all normal tissues. Fresh specimens of the resting breast, which were stained with polychrome methylene blue, and fixed specimens which were stained with hematoxylin and eosin, rarely showed nucleoli. On the contrary, the nucleoli were conspicuous in most of the cells of other tissues studied, particularly in fresh tissues. Normal cells usually contained a single, round, well-

defined nucleolus which appeared very small in relation to the nucleus. Occasionally, two or three nucleoli were seen within the nucleus, particularly in the normal stomach and rectum. The cells of inflammatory tissues and benign tumors almost always contained round or elliptic nucleoli. The size of the nucleoli depended on the type of the cell (primary or secondary cytoplasia of MacCarty), but the nucleoli almost always were larger than those of the corresponding normal tissue. The greatest variation in size, form, and number of the nuclei and nucleoli was seen in the cells of secondary cytoplasia and in those of cancer. In these cells two or three nucleoli frequently were seen within the nucleus. Occasionally, as many as six could be counted. These nucleoli usually were very large in relation to the nucleus; they were round or elliptic, and occasionally were elongated or somewhat triangular. When the nucleoli were multiple, one usually was much larger than the others.

The results of the biometric studies are reported separately for each organ.

Thyroid Gland.—In nonmalignant lesions of the thyroid gland the average areas of the nuclei and nucleoli were 28.13 and 1.08 square microns, respectively, and the average nucleonucleolar ratio was 30.4 : 1. In malignant lesions the average areas of the nuclei and nucleoli were 51.7 and 4.29 square microns, respectively, and the average nucleonucleolar ratio was 13.5 : 1. In carcinoma the areas of the nuclei varied from 32.45 to 76.0 square microns, and the areas of the nucleoli varied from 1.81 to 8.9 square microns. The nucleonucleolar ratio varied from 6.7:1 to 22.4:1. In all nonmalignant lesions, except exophthalmic goiter, the average areas of the nuclei and nucleoli were smaller than the smallest areas of the nuclei and nucleoli in carcinoma. In exophthalmic goiter the average areas of the nuclei and nucleoli were larger than the smallest areas of the nuclei and nucleoli in carcinoma. In thyroiditis and colloid adenoma the average nucleonucleolar ratio was more than three times as great as it was in carcinoma. In the fetal thyroid gland and in exophthalmic goiter the average nucleonucleolar ratio was less than the largest nucleonucleolar ratio in carcinoma.

The areas of the nuclei and nucleoli and the nucleonucleolar ratio in the different grades of carcinoma of the thyroid gland are shown in Table I. This table is of interest mainly because it shows that the average area of the nucleoli increases gradually and the nucleonucleolar ratio decreases as the grade of malignancy increases.

TABLE I

AREAS OF NUCLEI AND NUCLEOLI AND THE NUCLEONUCLEOLAR RATIO IN DIFFERENT GRADES OF CARCINOMA OF THE THYROID GLAND

GRADE OF CARCINOMA	CASES	AREA, SQUARE MICRONS						NUCLEONUCLEOLAR RATIO		
		NUCLEI			NUCLEOLI			SMALLEST	LARGEST	AVERAGE
		SMALLEST	LARGEST	AVERAGE	SMALLEST	LARGEST	AVERAGE			
1	3	40.8	72.1	53.0	1.8	3.3	2.4	21.3:1	22.4:1	21.8:1
2	5	36.4	69.4	47.5	2.1	5.9	3.7	10.4:1	16.3:1	12.5:1
3	5	45.2	66.3	56.3	3.4	6.7	5.0	9:1	13.2:1	11.5:1
4	5	32.4	76.0	49.9	3.6	8.9	5.9	6.7:1	9.2:1	8.3:1
	18			51.7			4.2			13.5:1

Breast.—In the study of the breast the results were rather similar to those of the study of the thyroid gland. In nonmalignant lesions of the breast the average areas of the nuclei and nucleoli were 29.87 and 1.26 square microns, respectively, and the average nucleonucleolar ratio was 23.7:1. In carcinoma of the breast the average areas of the nuclei and nucleoli were 54.81 and 4.57 square microns, respectively, and the nucleonucleolar ratio was 13.7. Examination of inflammatory lesions that were associated with primary cytoplasmia revealed that the average areas of the nuclei and nucleoli were considerably smaller than the smallest areas of the nuclei and nucleoli in carcinoma. The average nucleonucleolar ratio in these inflammatory lesions was almost twice as large as the largest nucleonucleolar ratio in carcinoma (36.6:1 and 20.8:1). On the contrary, in inflammatory lesions that were associated with secondary cytoplasmia the average area of the nucleoli closely approximated the smallest area of the nucleoli in carcinoma (2.04 and 2.2 square microns), and the average nucleonucleolar ratio was much lower than the largest ratio in carcinoma (15.8:1 and 20.8:1) and closely approximated the average nucleonucleolar ratio in carcinoma (15.8:1 and 13.7:1). In benign tumors that were associated with primary or secondary cytoplasmia the average areas of the nuclei and nucleoli were somewhat smaller than the smallest areas of the nuclei and nucleoli in carcinoma. In benign tumors in which there was primary cytoplasmia the average nucleonucleolar ratio was much larger than the largest ratio in carcinoma (30.4:1 and 20.8:1), but in benign tumors in which there was secondary cytoplasmia the average nucleonucleolar ratio was slightly smaller than the largest ratio in carcinoma (19.3:1 and 20.8:1). In carcinoma the areas of the nuclei ranged from 37.32 to 93.89 square microns, the areas of the nucleoli ranged from 2.2 to 13.48 square microns, and the nucleonucleolar ratio varied from 4.6:1 to 20.8:1 (Table II).

TABLE II

AREAS OF NUCLEI AND NUCLEOLI AND THE NUCLEONUCLEOLAR RATIO IN DIFFERENT GRADES OF CARCINOMA OF THE BREAST

GRADE OF CARCINOMA	CASES	AREA, SQUARE MICRONS						NUCLEONUCLEOLAR RATIO		
		NUCLEI			NUCLEOLI			SMALLEST	LARGEST	AVERAGE
		SMALLEST	LARGEST	AVERAGE	SMALLEST	LARGEST	AVERAGE			
1	5	40.9	57.0	49.1	2.3	3.0	2.6	15.1	20.8:1	18.4:1
2	6	39.6	56.8	47.2	2.2	5.1	3.3	10.9:1	17.8:1	14.7:1
3	5	37.3	93.8	66.1	4.2	6.1	5.6	6.1:1	14.9:1	11.8:1
4	5	52.4	72.7	57.0	4.1	13.4	6.6	4.6:1	13.2:1	10.1:1
	21			54.8			4.5			13.7:1

The results of biometric study of carcinoma of the breast were similar to those obtained in the study of carcinoma of the thyroid gland (Table I). The area of the nuclei did not have any relation to the grade of carcinoma, but the average area of the nucleoli increased gradually and the average nucleonucleolar ratio decreased as the grade of malignancy increased.

Stomach.—In nonmalignant lesions of the stomach the average areas of the nuclei and nucleoli were 38.2 and 1.8 square microns, respectively, and the

average nucleonucleolar ratio was 21.2:1. In carcinoma the average areas of the nuclei and nucleoli were 52.6 and 3.9 square microns, respectively, and the average nucleonucleolar ratio was 13.4:1. The areas of the nuclei and nucleoli of normal gastric tissue were rather constant and were much smaller than the respective areas in carcinoma (Fig. 1). The average nucleonucleolar ratio of



Fig. 1.

Fig. 1.—Cells of normal gastric mucosa. (Photomicrograph kindly loaned by Dr. W. C. MacCarty.)



Fig. 2.

Fig. 2.—Primary cytoplasia in the edges of a gastric ulcer. (Photomicrograph kindly loaned by Dr. W. C. MacCarty.)

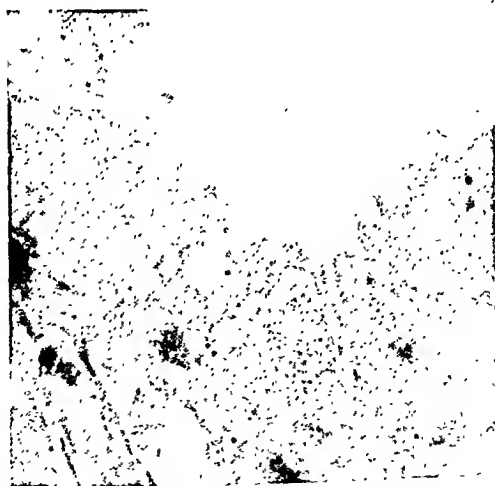


Fig. 3.—Secondary cytoplasia in the edges of a gastric ulcer. (Photomicrograph kindly loaned by Dr. W. C. MacCarty.)

normal gastric tissue was almost three times as large as it was in carcinoma (36.2:1 and 13.4:1). In fetal gastric tissue the average area of the nuclei was about the same as it was in carcinoma, the average area of the nucleoli was slightly larger than the smallest area of the nucleoli in carcinoma, and the nucleonucleolar ratio occupied an intermediate position between the ratios obtained in a study of normal gastric tissue and carcinoma of the stomach. Bio-

metric studies of inflammatory lesions that were associated with primary cytoplasia revealed that the nucleoli were somewhat larger than those found in normal gastric tissue (Fig. 2); the average nucleonucleolar ratio was similar to that obtained in a study of fetal gastric tissue. The material included only one benign tumor of the stomach that was associated with primary cytoplasia. In this tumor the nuclei and nucleoli were larger than those in normal gastric tissue, but the average nucleonucleolar ratio was slightly smaller than that of the normal gastric tissue. In benign tumors that were associated with secondary cytoplasia the measurements usually corresponded very closely to those obtained in grade 2 carcinoma. The same was true of gastric ulcers that were associated

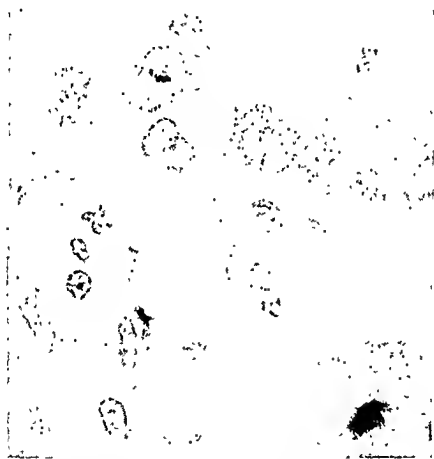


FIG. 4.—Carcinoma, grade 2, of the stomach ($\times 1,000$).

with secondary cytoplasia (Fig. 3). In carcinoma the smallest areas of the nuclei and nucleoli were 36.2 and 2.3 square microns, respectively, and the smallest nucleonucleolar ratio was 9.9:1. The largest areas of the nuclei and nucleoli were 74.9 and 6.7 square microns, respectively, and the largest nucleonucleolar ratio was 17.6:1 (Table III). The gastric material did not include a specimen of grade 1 carcinoma. A study of the material revealed that in carcinoma of the stomach the average area of the nucleoli increases and the nucleonucleolar ratio decreases as the grade of malignancy increases. The cells encountered in the different grades of carcinoma are shown in Figs. 4, 5, and 6.

Liver.—The biometric studies of the liver were of particular interest as there was a slight difference in the nucleonucleolar ratios in all lesions, including carcinoma. In nonmalignant lesions of the liver the average areas of the nuclei

and nucleoli were 33.0 and 1.9 square microns, respectively, and the average nucleonucleolar ratio was 16.9:1. In malignant lesions the average areas of the nuelei and nucleoli were 64.6 and 6.1 square microns, respectively, and the aver-

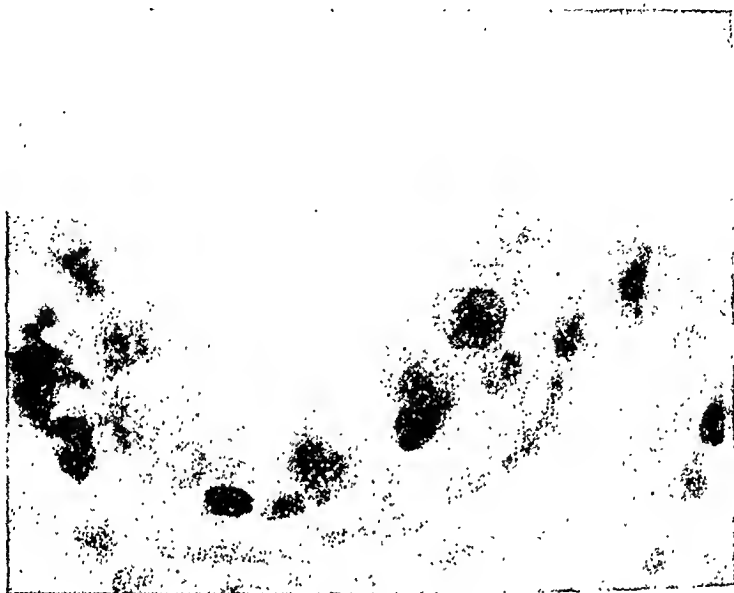


Fig. 5.—Carcinoma, grade 3, of the stomach ($\times 1,000$).



Fig. 6.—Carcinoma, grade 4, of the stomach ($\times 1,000$).

age nucleonucleolar ratio was 11.6:1. In the fetal liver the nuelei and nucleoli were small in comparison with the very large nuelei and nucleoli in carcinoma. However, the nucleonucleolar ratio was not much higher than was the average ratio in all grades of carcinoma (14.1:1 and 11.6:1); in fact, the average nucleo-

TABLE III

AREAS OF NUCLEI AND NUCLEOLI AND THE NUCLEONUCLEOLAR RATIO IN DIFFERENT GRADES OF CARCINOMA OF THE STOMACH

GRADE OF CARCINOMA	CASES	AREA, SQUARE MICRONS						NUCLEONUCLEOLAR RATIO		
		NUCLEI			NUCLEOLI			SMALLEST	LARGEST	AVERAGE
		SMALLEST	LARGEST	AVERAGE	SMALLEST	LARGEST	AVERAGE			
1	0									
2	8	36.8	66.0	48.8	2.3	5.3	3.2	12.4:1	17.6:1	15.3:1
3	5	38.8	73.6	52.3	3.0	4.8	3.8	11.2:1	17:1	13.3:1
4	5	36.2	74.9	56.7	2.7	6.7	4.9	9.9:1	13.7:1	11.8:1
	18			52.6			3.9			13.4:1

nucleolar ratio in the fetal liver was higher than the smallest ratio in grade 2 carcinoma (15.4:1 and 14.1:1). In normal hepatic tissue the nuclei and nucleoli varied a great deal in size, but they were rather large. The average nucleonucleolar ratio was low; it was only 6.1 more than the average ratio in carcinoma and only 2.3 greater than the average ratio in grade 2 carcinoma. In inflammatory lesions the nuclei and nucleoli were moderately large, and the nucleonucleolar ratio was low (19.0:1), but contrary to what was found in most of the other organs that were studied, the nucleonucleolar ratio was larger than that encountered in normal hepatic tissue. In carcinoma of the liver the areas of the nuclei varied from 42.5 to 88.4 square microns, the areas of the nucleoli varied from 3.0 to 10.6 square microns, and the nucleonucleolar ratio varied from 6.7:1 to 18.1:1 (Table IV). The hepatic material did not include a specimen of

TABLE IV

AREAS OF NUCLEI AND NUCLEOLI AND THE NUCLEONUCLEOLAR RATIO IN DIFFERENT GRADES OF CARCINOMA OF THE LIVER

GRADE OF CARCINOMA	CASES	AREA, SQUARE MICRONS						NUCLEONUCLEOLAR RATIO		
		NUCLEI			NUCLEOLI			SMALLEST	LARGEST	AVERAGE
		SMALLEST	LARGEST	AVERAGE	SMALLEST	LARGEST	AVERAGE			
1	0									
2	3	42.5	59.2	49.6	3.0	3.3	3.2	14.1:1	18.1:1	15.4:1
3	4	68.6	88.4	79.0	6.2	7.1	6.7	10.7:1	13.1:1	11.6:1
4	5	55.9	75.2	65.3	5.9	10.6	8.5	6.7:1	9.3:1	7.8:1
	12			64.6			6.1			11.6:1

grade 1 carcinoma. A study of the material revealed that the average area of the nucleoli increases and the nucleonucleolar ratios decrease as the grade of malignancy increases. There was no relation between the areas of the nuclei and the grade of carcinoma.

Rectum.—In nonmalignant lesions of the rectum the average areas of the nuclei and nucleoli were 40.6 and 1.8 square microns, respectively, and the average nucleonucleolar ratio was 22.5:1. In carcinoma the average areas of the nuclei and nucleoli were 62.6 and 7.5 square microns, respectively, and the average nucleonucleolar ratio was 11.0:1 (Table V). A study of the fetal rectum

TABLE V

AREAS OF NUCLEI AND NUCLEOLI AND THE NUCLEONUCLEOLAR RATIO IN DIFFERENT GRADES OF CARCINOMA OF THE RECTUM

GRADE OF CARCINOMA	CASES	AREA, SQUARE MICRONS						NUCLEONUCLEOLAR RATIO		
		NUCLEI			NUCLEOLI			SMALLEST	LARGEST	AVERAGE
		SMALLEST	LARGEST	AVERAGE	SMALLEST	LARGEST	AVERAGE			
1	5	48.0	72.4	57.0	3.0	5.1	3.8	12.4:1	18.3:1	14.9:1
2	5	36.2	56.4	43.1	2.6	4.9	3.7	11.2:1	14.1:1	12.2:1
3	5	54.6	81.0	69.2	7.1	8.7	7.9	6.8:1	11.4:1	8.7:1
4	4	41.5	174.3	80.9	4.0	32.5	14.6	5.3:1	10.4:1	8.4:1
	19			62.6			7.5			11:1

disclosed that the nucleoli were relatively large; in fact, they were almost as large as the smallest nucleoli in carcinoma. The nuclei also were large and the nucleonucleolar ratio was high. The material included two inflammatory lesions of the rectum. Primary cytoplasmia was present in both of these lesions. The nuclei and nucleoli were small, and the average nucleonucleolar ratio was about twice as large as the ratio in carcinoma. In a benign polyp in which there was primary cytoplasmia the measurements were very much like those of the inflammatory lesions. The results of biometric studies of the rectum were slightly different from the results of similar studies of other organs. In grade 2 carcinoma, the average area of the nucleoli was 0.1 square micron less than it was in grade 1 carcinoma. However, in comparison with the average area of nucleoli in grade 3 carcinoma, that of nucleoli in grade 4 carcinoma was increased markedly; in fact, in each of these grades of carcinoma the average area of the nucleoli was almost twice as great as it was in the next lower grade. In all grades of carcinoma the nucleonucleolar ratio decreased as the grade of malignancy increased, but in grade 3 carcinoma and in grade 4 carcinoma the difference in the ratio was very slight.

Ovary.—In nonmalignant lesions of the ovary the average areas of the nuclei and nucleoli were 41.0 and 2.0 square microns, respectively, and the average nucleonucleolar ratio was 20.5:1. In carcinoma the average areas of the nuclei and nucleoli were 48.7 and 4.0 square microns, respectively, and the aver-

TABLE VI

AREAS OF NUCLEI AND NUCLEOLI AND THE NUCLEONUCLEOLAR RATIO IN DIFFERENT GRADES OF CARCINOMA OF THE OVARY

GRADE OF CARCINOMA	CASES	AREA, SQUARE MICRONS						NUCLEONUCLEOLAR RATIO		
		NUCLEI			NUCLEOLI			SMALLEST	LARGEST	AVERAGE
		SMALLEST	LARGEST	AVERAGE	SMALLEST	LARGEST	AVERAGE			
1	2	25.4	32.0	28.7	1.9	2.3	2.1	12.9:1	13.9:1	13.4:1
2	3	41.0	80.3	58.4	2.5	7.5	4.7	10.7:1	15.8:1	13.4:1
3	3	42.6	71.5	54.0	3.8	5.2	4.6	10:1	13.6:1	11.5:1
4	0						4.0			12.3:1
	8			48.7						

age nucleonucleolar ratio was 12.3:1 (Table VI). The size of the cells of the membrana granulosa of a Graafian follicle is shown in Fig. 7. In the theca interna of a Graafian follicle the nuclei were very large and the nucleoli also were large; the nucleonucleolar ratio was almost twice as large as it was in carcinoma. In one ovary the average area of the nucleoli of the membrana granulosa was slightly less than the average area of the nucleoli of the theca interna, but the average area of the nuclei of the membrana granulosa was about half as large as the average area of the nuclei in the theca interna. The average nucleonucleolar ratio in the membrana granulosa therefore was low. It was not much higher than the average ratio in carcinoma (14.0:1 and 12.3:1) and was lower than the



Fig. 7.—Membrana granulosa of a Graafian follicle ($\times 1,000$).

ratio in certain specimens of carcinoma. In the corpus luteum the measurements were similar to those obtained for the theca interna; however, in the corpus luteum the average area of the nuclei and, therefore, the average nucleonucleolar ratio, were somewhat lower than they were in the theca interna. In a benign papillary cyst the average areas of the nuclei and nucleoli were very small, and the average nucleonucleolar ratio was about three times as large as it was in carcinoma. In carcinoma of the ovary (Table VI) the measurements were not entirely in agreement with the measurements obtained in specimens of carcinoma of other organs, but the differences were not very marked. As was the case in carcinoma of other organs, there was no relation between the average area of the nuclei and the grade of carcinoma.

Endometrium.—In benign lesions of the endometrium the average areas of the nuclei and nucleoli were 30.68 and 0.84 square microns, respectively, and the average nucleonucleolar ratio was 36.5:1. In carcinoma the average areas of the nuclei and nucleoli were 55.5 and 4.7 square microns, respectively, and the average nucleonucleolar ratio was 13.4:1 (Table VII). Normal endometrial tissue had small nuclei, very small nucleoli, and a very high nucleonucleolar ratio. In inflammatory lesions in which there was primary cytoplasmia the meas-

TABLE VIII

AREAS OF NUCLEI AND NUCLEOLI AND THE NUCLEONUCLEOLAR RATIO IN DIFFERENT GRADES OF CARCINOMA OF THE ENDOMETRIUM

GRADE OF CARCINOMA	CASES	AREA, SQUARE MICRONS						NUCLEONUCLEOLAR RATIO		
		NUCLEI			NUCLEOLI					
		SMALLEST	LARGEST	AVERAGE	SMALLEST	LARGEST	AVERAGE	SMALLEST	LARGEST	AVERAGE
1	6	31.0	65.5	45.3	1.7	4.7	2.6	13.9:1	23.7:1	18.6:1
2	6	45.2	63.0	52.4	3.1	4.1	3.4	11.8:1	17.8:1	15.1:1
3	1			51.5			4.5			11.4:1
4	1			72.6			8.2			8.8:1
	14			55.5			4.7			13.4:1

urements approximated those obtained for normal endometrial tissue; the nuclei and nucleoli were smaller, but the nucleonucleolar ratio was slightly higher. In a polypoid endometrium the nuclei also were smaller than they were in normal endometrial tissue, but the average area of the nucleoli was larger than it was in the normal endometrium. The average nucleonucleolar ratio, therefore, was much smaller than the ratio in the normal endometrium, but it was more than twice as large as the average ratio in carcinoma. In a benign polyp the measurements were similar to those obtained for the normal endometrium, but the average nucleonucleolar ratio was a little lower. The number of carcinomas of the endometrium was very small; only one carcinoma was classified in each of the two highest grades of malignancy (grades 3 and 4). However, the results of the biometric studies were similar to the results obtained in the study of other organs; the average area of the nucleoli gradually increased, and the nucleonucleolar ratio decreased as the grade of malignancy increased.

Uterine Cervix.—In nonmalignant lesions of the uterine cervix the average areas of the nuclei and nucleoli were 30.97 and 0.78 microns, respectively, and the nucleonucleolar ratio was 32.7:1. In carcinoma the average areas of the nuclei and nucleoli were 63.7 and 7.0 square microns, respectively, and the average nucleonucleolar ratio was 10.1:1 (Table VIII). Biometric studies of inflammatory lesions revealed that the average areas of the nuclei and nucleoli were large, and the average nucleonucleolar ratio was more than three times as large as it

TABLE VIII

AREAS OF NUCLEI AND NUCLEOLI AND THE NUCLEONUCLEOLAR RATIO IN DIFFERENT GRADES OF CARCINOMA OF THE UTERINE CERVIX

GRADE OF CARCINOMA	CASES	AREA, SQUARE MICRONS						NUCLEONUCLEOLAR RATIO		
		NUCLEI			NUCLEOLI					
		SMALLEST	LARGEST	AVERAGE	SMALLEST	LARGEST	AVERAGE	SMALLEST	LARGEST	AVERAGE
1	0									
2	2	54.4	69.4	61.9	4.2	6.3	5.3	8.6:1	16.4:1	12.5:1
3	4	57.3	79.5	66.3	5.1	13.9	9.7	5.7:1	12.7:1	7.7:1
4	3	55.0	59.1	62.9	5.3	6.7	6.0	9.3:1	12.2:1	10.3:1
	9			63.7			7.0			10.1:1

was in carcinoma. In a benign polyp the nuelei were large but the nucleoli were small; the nucleonucleolar ratio was about as large as it was in normal tissue. In the biometric studies of carcinoma of the cervix the results were entirely different from the results of similar studies of carcinoma of other organs.

COMMENT

In carcinoma of practically every organ there was some overlapping of the areas of the nucleoli and the nucleonucleolar ratios. However, the overlapping usually was seen between grades 1 and 2, 2 and 3, or 3 and 4; it very rarely was noted between grades 1 and 3, 1 and 4, or 2 and 4.

A careful examination of the results and the tables seems to leave no doubt that, in general, no matter what organ is considered, the average areas of the nucleoli in relation to those of the nuelei are absolutely and relatively much larger in carcinomas than they are in benign lesions. According to this, the average nucleonucleolar ratios are much smaller in carcinoma than they are in benign lesions.

There were few individual specimens of nonmalignant lesions in which the nucleonucleolar ratio was almost as low or even as low as it was in carcinoma. This happened in inflammatory lesions and in benign tumors in which there was secondary cytoplasmia. It is known how frequently certain benign tumors and chronic ulcers become malignant; the ulcers and tumors that become malignant probably are those that are associated with secondary cytoplasmia. In the edges of chronic gastric ulcers and in some of the acini of benign epithelial tumors, the cells of secondary cytoplasmia repeatedly have been seen passing through the basal membrane and invading the tissues in an area that histologically looked perfectly benign and had been previously diagnosed as such. The reverse is also true, particularly in carcinoma of the stomach. In the transitional zone of the mucosa where the growth stops and the normal or inflammatory mucosa begins, the mucosa looks histologically normal for a more or less long distance. However, in the same zone the microscopic examination with an oil-immersion objective shows that the acini are filled with cells of secondary cytoplasmia, and occasionally that one or more acini are broken. This permits the cells to pass into the stroma. The nucleonucleolar ratio of the cells of secondary cytoplasmia in this transitional zone is practically the same as it is in the carcinoma near by.³³ The cells of secondary cytoplasmia probably are cancer cells which have not yet invaded the stroma for some unknown reason. MacCarty,²⁶ Broders,⁴ Raaf,³⁷ and others have expressed the same opinion. Broders⁴ went so far as to consider the cells of secondary cytoplasmia as cancer cells within the tubules or acini. Other cells in which the nucleonucleolar ratios approximate the ratios in carcinoma are those of the membrana granulosa of the ovary, fetus, normal liver, and exophthalmic goiter. The metabolic activity of the cells of these tissues is well known. In addition, at least in the fetus and exophthalmic goiter, there is no doubt that the cells grow fast and have a great reproductive power. Therefore, it seems logical to admit that the large amount of nucleolar substance and the low nucleonucleolar ratio in the nonmalignant lesions that were studied is somewhat related to the great metabolic activity and possibly also to the rapid growth and cellular division. This is not a new idea; the experimental work on

lower animals of Wace, Ludford, and Sayles seems to prove this. Sayles said: "The amount of nucleolar material present in a cell seems to be an index of the activity of its nucleus both in cell metabolism and in preparation for cell division. Two nucleoli within a single nucleus are the result of an increase in nucleolar substance beyond the amount which can exist within the nucleus as a single droplet." The conclusions of Ludford are also conclusive since he said: "Actively growing cells are characterized by their large nucleoli, and by the fragmentation and division of the same. In the adult, nucleoli of cells undergoing active metabolism are relatively large, those in 'resting condition relatively small.'"

It is generally admitted that cancer cells are metabolically very active and have a great reproductive power. Fischer said that in the cultures of cancer cells there are found ten times as many mitotic figures as in the cultures of normal tissues. Since cultures of cancer cells grow slower than those of normal tissues, he explained this fact by the necessity of the cancer cells to divide quickly and said that many divide at one time in order to compensate for the loss of substance produced by the disintegration of many cancer cells whose life has a short duration as has been proved in growth of carcinomatous tissues "in vitro." According to Broders and others, both metabolic activity and reproductive power of the cells in carcinoma increase from grade 1 to grade 4. On the other hand, in Tables I, II, III, and IV, it may be seen that the average area of the nucleoli increases and the average nucleonucleolar ratio decreases in each grade from grade 1 to grade 4. This seems to prove that in malignant lesions the size of the nucleoli also is in direct relation with the degree of malignancy, or better, the nucleonucleolar ratio is in inverse relation with the degree of malignancy. The results shown in Table V corroborate this opinion. Although there is a slight decrease of the average area of the nucleoli from grade 1 to grade 2, the average nucleonucleolar ratio decreases in every grade from grade 1 to grade 4. The results shown in Table VI are not in agreement with this opinion, but they should not be considered when discussing averages; the number of carcinomas of the ovary in this series was very small. The same should be said for the results shown in Table VII, although these corroborate my opinion. For the same reason, the results shown in Table VIII will not be discussed. Even if this table had included a large series of cases the results should not be considered because most of the carcinomas of the cervix are treated by radium before operation is performed; this alters structures, metabolic cellular activity, and growth.

The following questions might arise: (1) If the amount of nucleolar material increases with the grade of carcinoma, or rather, if the nucleonucleolar ratio decreases according to the grade of carcinoma, how shall the overlapping from grade to grade be explained? (2) Why do certain grade 1 carcinomas, in the same organ have the same nucleonucleolar ratio as some grade 2 carcinomas? (3) In the same organ, why is the nucleonucleolar ratio in grade 2 carcinoma the same as in grade 3 carcinomas, and occasionally even in grade 4 carcinomas? The answers to these questions can be only the following ones: (1) The grading of carcinomas depends to a certain extent upon personal estimation of the pathologist who did the grading. (2) The grading of carcinoma has value for orientation of treatment and prognosis but mainly when considered for a group of cases. For some unknown cause the cells of a grade 2 carcinoma may at any

time become very active and soon behave as a grade 3 or 4 carcinoma. On the contrary the cells of a grade 3 or 4 carcinoma may possibly become inactive, and the tumor thus become quiescent.

The relation of the nucleonucleolar ratio to cellular activity being admitted, the lack of constancy of that ratio that has been found by different authors, or even by the same author on different occasions, in normal and pathologic tissues of the same kind can be understood. Thus, for example, in malignant lesions of the stomach the discrepancy of results found by Haumeder (9:1), Naidu (16:1), and myself (14.9:1) is probably due to the fact that in the series of cases studied by Haumeder there were more carcinomas of a high grade than there were in the series studied by Naidu and myself.

CONCLUSIONS

1. The average areas of the nuclei and nucleoli, and the average nucleonucleolar ratio, are not the same in all normal tissues; they vary, probably, according to the function of the organ, and in the same organ they vary with the metabolic activity.

2. In nonmalignant lesions the average area of the nucleoli is smaller and the average nucleonucleolar ratio is larger than in carcinoma.

3. In the cells of the fetus, normal liver, membrana granulosa of the ovary, and exophthalmic goiter the areas of nucleoli and the nucleonucleolar ratios approximate those found in carcinoma. This is probably attributable to the great metabolic activity of these cells.

4. There is more variation in the extreme areas of the nuclei and nucleoli in carcinoma than there is in nonmalignant lesions.

5. The average area of the nucleoli and the average nucleonucleolar ratio vary according to the degree of malignancy; the former in a direct and the latter in an inverse proportion. In carcinoma the average area of the nucleoli increases and the average nucleonucleolar ratio decreases from grade 1 to grade 2, from grade 2 to 3, and from 3 to grade 4.

6. The increased amount of nucleolar substance in cancer cells is most probably attributable to the great cellular metabolism and cellular division characteristic of those cells.

7. The increasing of nucleolar substance associated with the increasing in the grade of carcinoma is most probably attributable to the increase of cellular metabolism and cellular division from grade 1 to 2, from grade 2 to 3, and from grade 3 to 4.

REFERENCES

1. Bennett: Quoted by Lebert.
2. Broders, A. C.: Squamous-Cell Epithelioma of the Lip. A Study of 537 Cases, J. A. M. A. 74: 656, 1920.
3. Broders, A. C.: The Grading of Carcinoma, Minnesota Med. 8: 726, 1925.
4. Broders, A. C.: Cancer's Self-control, M. J. & Record 121: 133, 1925.
5. Broders, A. C.: Carcinoma, Grading and Practical Application, Arch. Path. & Lab. Med. 2: 376, 1926.
6. Campbell, S. J.: Quoted by MacCarty, W. C.³⁰
7. Castron, H.: Quoted by Von Haam, E., and Alexander, H. G.
8. Cohen, M. F.: Quoted by MacCarty, W. C.³⁰

9. Ehrlich, W. E.: Nuclear Sizes in Growth Disturbances; With Special Reference to the Tumor Cell Nucleus, *Am. J. M. Sc.* 192: 772, 1936.
10. Fairchild, R. D.: Quoted by MacCarty, W. C.³⁰
11. Farthing, J. W.: Quoted by MacCarty, W. C.³⁰
12. Fidler, H. K.: A Comparative Cytological Study of Benign and Malignant Tissues, *Am. J. Cancer* 25: 772, 1935.
13. Fischer, Albert: La physiologie de la cellule cancéreuse, *Cancer, Bruxelles* 12: 160, 1935.
14. Frugoni, P.: Quoted by MacCarty, W. C.³⁰
15. Haumeder, Eva: Vergleichende Kern- und Nucleolermessungen an verschiedenen Organen mit besonderer Berücksichtigung der malignen Tumorzellen, *Ztschr. f. Krebsforsch* 40: 105, 1933.
16. Karp, Herbert: Cytodiagnostik maligner Tumoren aus Punktaten und Sekreten, *Ztschr. f. Krebsforsch.* 36: 579, 1932.
17. Kaump, D. H., and Mendes Ferreira, A. E.: Papillomas of the Breast. Study of 273 Specimens, *J. LAB. & CLIN. MED.* 22: 681, 1937.
18. Kroeze, R. G.: Quoted by MacCarty, W. C.³⁰
19. Lebert, Hermann: *Traité pratique des maladies cancéreuses et des affections curables condondues avec le cancer*, Paris, 1851, J. B. Baillière, 892, pp.
20. Lofgren, R. C.: Quoted by MacCarty, W. C.³⁰
21. Ludford, R. J.: V. The Morphology and Physiology of the Nucleolus. Part I. The Nucleolus in the Germ-Cell Cycle of the Mollusc stagnalis, *J. Roy. Micr. Soc.* p. 113, 1922.
22. MacCarty, W. C.: The Cytological Diagnosis of Neoplasms, *J. A. M. A.* 81: 519, 1923.
23. MacCarty, W. C.: The Early Diagnosis of Cancer, *Arch. Clin. Cancer. Res.* 1: 11, 1925.
24. MacCarty, W. C.: A Cytologic Key to the Diagnosis and Prognosis of Neoplasm, *J. LAB. & CLIN. MED.* 13: 354, 1928.
25. MacCarty, W. C.: The Diagnostic Reliability of Frozen Sections, *Am. J. Path.* 5: 377, 1929.
26. MacCarty, W. C.: The Malignant Cell, *J. Cancer Research* 13: 167, 1929.
27. MacCarty, W. C.: Principles of Prognosis in Cancer, *J. A. M. A.* 96: 30, 1931.
28. MacCarty, W. C.: Identification of the Cancer Cell, *J. A. M. A.* 107: 844, 1936.
29. MacCarty, W. C.: Microscopic Grading of Tumors, Its Interpretation, Limitations, and Relation to Radiosensitivity, *Am. J. Roentgenol.* 37: 365, 1937.
30. MacCarty, W. C.: Further Observations on the Macronucleolus of Cancer, *Am. J. Cancer* 31: 104, 1937.
31. MacCarty, W. C., Haumeder, Eva, and Berkson, Joseph: A Differential Characteristic of Malignant Cells: Preliminary Report, *Proc. Staff Meet., Mayo Clin.* 8: 38, 1933.
32. McCormack, C. J.: Biometric Studies of Endometrial Cells, *Proc. Staff Meet., Mayo Clin.* 10: 24, 1935.
33. Mendes Ferreira, A. E.: A Cytological Study of Carcinoma of the Stomach. Unpublished data.
34. Naidu, V. R.: The Value of Enlarged Nucleoli in the Diagnosis of Malignancy, *Proc. Staff Meet., Mayo Clin.* 10: 356, 1935.
35. Page, R. C.: Quoted by MacCarty, W. C.³⁰
36. Quensel, Ulrik: Zytologische Untersuchungen von Ergüssen der Brust- und Bauchhöhlen mit besonderer Berücksichtigung der karzinomatösen Exsudate, Uppsala, 1925, Almqvist & Wiksells; *Acta. med. Scandinav.* (suppl. 23) 68: 108, 1928.
37. Raaf, J. E.: A Study of Atypical Cellular Hyperplasia in Gastric Ulcers and Its Relationship to Cancer, *Proc. Staff Meet., Mayo Clin.* 10: 641, 1935.
38. Sayles, L. P.: Origin of the Mesoderm and Behavior of the Nucleolus in Regeneration in Lumbriculus, *Biol. Bull.* 52: 278, 1927.
39. Saxen, Arno: Quoted by Von Haam, E., and Alexander, H. G.
40. Smith, C. H.: Quoted by MacCarty, W. C.³⁰
41. Stenius, Fjalar: Zur Bewertung des epithelialen Harnsedimentes für die Diagnose der Blasengeschwülste, *Acta chir. Scandinav.* 58: 240, 1925.
42. Strohl, E. L.: Cytologic Study of a Suprarenal Tumor, *Proc. Staff Meet., Mayo Clin.* 10: 353, 1935.
43. Virchow, R.: Die endogene Zellenbildung beim Krebs, *Virchows Arch. f. path. Anat.* 3: 197, 1851.
44. Vögel: Quoted by Lebert, Hermann.
45. Von Haam, E., and Alexander, H. G.: Cytological Studies of Malignant Tumors, *Am. J. Clin. Path.* 6: 394, 1936.
46. Wace, C. E.: Quoted by Ludford, R. J.

TOXIC REACTIONS WITH GOLD SALTS IN TREATMENT OF RHEUMATOID ARTHRITIS*

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IN ANY disease where new remedies are being introduced at frequent intervals one can be sure that none of the proposed methods has a generally accepted value. This condition has prevailed particularly in the treatment of rheumatoid arthritis where an unusually large number of preparations have been suggested. The typical course for such a "new" treatment, as stated by Hench and others¹ is that it "... rises rather rapidly, reaches its peak in about 3 to 5 years, then falls as adverse reports begin to outnumber the optimistic ones. Finally, use of the treatment in any significant degree dies out after 8 to 10 years." These same authors point out that it is significant that the curve of acceptance of gold salts in the treatment of rheumatoid arthritis is still rising after ten years of use.

Many enthusiastic reports on gold compounds in rheumatoid arthritis have been appearing in the European literature, and recently favorable reports have been made by American workers in this field. One of the earliest American reports is by Phillips,² who employed chrysotherapy in 18 patients, 9 of whom were diagnosed as having rheumatoid arthritis. Phillips was not favorably impressed with the value of gold salts. Shortly after this Oren³ gave his results with gold salts in 100 cases of arthritis. Of the 66 cases of rheumatoid arthritis in his series, 60 "responded well." Key and his co-workers⁴ have reported their results from the arthritis clinic of the Washington University School of Medicine, St. Louis. In 70 cases of rheumatoid arthritis they found gold salts definitely ameliorated the course of the disease in the majority of the patients, regardless of the duration of the disease. Sashin, Spanbock, and Kling⁵ found moderate to marked improvement in 85 per cent of the 80 patients with rheumatoid arthritis who received gold salts. Snyder, Traeger, and Kelly⁶ noted improvement in 48 per cent of the 50 cases of rheumatoid arthritis after chrysotherapy.

Those who have had experience with this form of therapy emphasize that, although we have in chrysotherapy a most effective method for the treatment of rheumatoid arthritis, the use of the gold preparations is not entirely without danger. Hartfall, Garland, and Goldie⁷ reported toxic reactions in 41.9 per cent of 900 patients treated with gold compounds. They had 7 deaths—a mortality rate of 0.78 per cent—definitely attributable to gold salts. Sixty-three per cent of the patients treated by Key⁴ had some form of reaction. Crosby⁸ has compared this group of reactions to the accidents that occurred in the early days of organic arsenical drugs.

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Chrysotherapy is constantly becoming more popular, and pharmaceutical houses are preparing advertising campaigns to acquaint physicians with the merits of their preparations. In view of the widespread interest that is being created by this type of treatment, it is of utmost importance that the physician recognize the different types of reaction to the drug and that the accumulated experience of previous workers be utilized in forming a basic plan of treatment. We have followed more than 100 clinic and private patients treated with gold compounds during the past two years, and it is on our experience with this group that this report is based.

SKIN REACTIONS

The most common toxic reactions due to gold preparations are those occurring in the skin. Pruritus is very frequent after chrysotherapy; it may be generalized or it may involve one or more localized parts of the body. Erythema, often accompanied by pruritus, is also a frequent complication. This, too, may be local or generalized in its distribution. One often encounters a lesion resembling lichen planus. There may be only one or two such areas, or there may be numerous patches scattered over the body. The lesions vary in size from a few millimeters in diameter to a patch having a dimension of 3 or 4 cm. In a few instances urticaria occurs.

Occasionally a patient will develop exfoliative dermatitis. This is always a serious complication; when the process is widespread, the patients are very susceptible to pneumonia.

The pruritus, erythema, and urticaria reactions generally clear shortly after stopping the gold compounds. The lesions resembling lichen planus not infrequently persist and spread for a period of several months after discontinuing therapy. The seriousness of exfoliative dermatitis seems to be in proportion to the extent of the lesion, and the treatment is the same as that of a similar lesion due to an arsenical or other agent. One of our patients developed a complete glove-like desquamation of the skin of both hands and recovered without the aid of any particular therapy.

LESIONS OF THE MOUTH

Second only to skin lesions in frequency are the reactions that occur in the mouth. The usual mouth involvement is a soreness that is noted especially in the tongue; at times the patient will complain of numbness of the tongue. Examination of the mouth at this time reveals no unusual findings. In other instances an ulcerative stomatitis occurs. This is very painful when the patient takes food. Occasionally there are small ulcerations of the buccal mucous membranes. After stopping therapy, the duration of mouth lesions is roughly in proportion to the severity of the involvement, the ulcerations persisting as long as two or three months.

GASTROINTESTINAL LESIONS

Disturbances of the gastrointestinal tract have been seen fairly often by some workers, but in our experience we have not found it a frequent complication of gold therapy. The patient who has a reaction involving the gastrointestinal tract will complain of cramps, nausea or vomiting, or diarrhea. These

symptoms may be mild or severe and may occur alone or in combination. Reactions of this type will clear promptly after the gold salts are stopped.

HEPATIC LESIONS

Jaundice of a benign type is seen at times. It is probably due to a toxic hepatitis and is very apt to run a lengthy course. Several fatal cases of acute yellow atrophy of the liver have been mentioned by others,⁸ but this type of reaction is very rare.

RENAL LESIONS

A mild albuminuria is a very frequent complication and is no indication for stopping the medication. Hematuria or a marked degree of albuminuria will disappear shortly after discontinuing treatment.

VASOMOTOR REACTIONS*

In almost every case the patient will experience a flushing of the face within one or two minutes after receiving the gold compound. The face appears fiery red, and the patient complains of a feeling of unusual warmth, often accompanied by a sensation of giddiness and vertigo. This reaction generally passes away in three or four minutes and leaves no aftereffect. One of our patients had such a reaction occurring about eight hours after he received the gold preparation. In another instance the vasomotor reaction became more severe with each subsequent treatment and the patient eventually had an attack of syncope. The patient collapsed and for several seconds the radial pulse could not be made out and the heart sounds were not audible. Stimulants were used and as the heart action resumed showers of ectopic beats were made out; in an hour's time the regular sinus rhythm was restored.

LOCAL REACTIONS

A large number of patients complain some time in the first two to six weeks of treatment that their arthritis is very much worse. Examination at this time will reveal that there has been a flare-up in joints in which the arthritis has been dormant and that there has been an increase of pain in the joints showing an active arthritis. Most of the patients experiencing this sort of reaction are likely to show a very satisfactory improvement with continued gold salt therapy. At times the exacerbation of joint symptoms will be accompanied by a rise of temperature to 102° or 103° F. In a few cases the temperature rise has occurred with no indication of joint or other reaction.

BLOOD DYSCRASIAS

It is in this group of reactions that fatalities are most likely to occur. Fortunately, reactions of this type are very infrequent. Cases of thrombocytopenic purpura, agranulocytosis, and aplastic anemia have been described, and in several instances a fatal outcome has resulted.

TIME OF ONSET OF REACTIONS

Except for the vasomotor disturbances it is impossible to predict the time of occurrence of reactions. Any of the reactions that have been described can oc-

*This type of reaction occurred principally with myochryslne. Recent change in the method of producing the preparation has practically eliminated this vasomotor effect.

cur after the first full dose of gold. Frequently a reaction will occur midway in a course of treatment. Many toxic reactions have occurred at a considerable time after the cessation of chrysotherapy. Reznikoff⁹ treated a young nurse for aplastic anemia five years after she had received gold salts for lupus vulgaris. It is the unpredictable nature of these reactions that constitutes the chief hazard in the use of gold salts in rheumatoid arthritis.

PREVENTION AND TREATMENT OF REACTIONS

Were it possible to prevent or minimize the reactions to gold salts we would have a potent remedy that could be employed easily by any physician. In an early paper in which they report on their first 100 cases of rheumatoid arthritis treated with gold salts, a statement made by Hartfall and Garland¹⁰ is still most pertinent: "Chrysotherapy should only be undertaken by those who are fully alive to its dangers. The patient should be warned of these possibilities."

Skin tests and patch tests have been of no value in indicating those who might have reactions. The simultaneous parental administration of sodium thiosulfate or of calcium preparations has not reduced the incidence of reactions. At times it has seemed that an increased vitamin C intake, such as can be obtained from the daily intake of several glasses of orange or tomato juice, lowered the number of reactions, but this has not been confirmed.

The reactions are treated by a method identical to that employed in similar lesions due to any other cause or factor. Nicotinic acid has been considered helpful by some in the treatment of the minor reactions; others have favored cevitamic acid; neither seems to alter the course of reactions to any significant degree. In exfoliative dermatitis sodium thiosulfate and calcium salts are recommended empirically.

CONTRAINDICATIONS

The only definite contraindications to the institution of gold salt therapy are blood dyscrasias and diseases of the kidneys or liver. Patients with hepatic or renal involvement, or who have had serious disturbance of the hematopoietic system are poor risks for this form of therapy. Gold compounds should be used with extreme caution in patients who give a personal or familial history of allergy. Treatment should be stopped at the first sign of any reaction, regardless of how trivial it may seem. When skin lesions, lesions of the mouth, gastrointestinal disturbances, and the less severe renal lesions have completely disappeared, treatment may be resumed. If any other type of reaction should occur, it is not advisable to resume gold therapy.

The presence of essential hypertension or of arteriosclerosis is no contraindication to this method of treatment.

CHOICE OF PREPARATIONS

There are several preparations of gold salts on the market; these differ in composition of the salt but all have in common the presence of a sulfur radical in the compound. There is a considerable difference of opinion in regard to variation in toxicity of the different preparations. Some of the patients who have toxic reactions appear to have an idiosyncrasy to gold and react to the first dose or to very small doses. Others seem to require a certain amount of

the gold salts before they have a similar reaction. In this latter group the problem arises whether it is safer to employ any particular compound.

We have preferred a watery solution of gold sodium thiomalate (myochrysin) given intragluteally. With this preparation we have had the usual minor toxic reactions and occasionally one of the more severe type. There have been no fatalities in this group. Gold sodium thiomalate is being used satisfactorily by many who are employing chrysotherapy.

In a very small series of patients who received gold sodium thiosulfate in our arthritis clinic at Bellevue Hospital, there have been two with very severe reactions, one of whom died. One of these patients developed an exfoliative dermatitis after receiving an initial dose of 0.05 Gm. of gold sodium thiosulfate intravenously, followed by 8 injections of 0.1 Gm. at weekly intervals—a total of 0.85 Gm. of the drug. She was admitted to the hospital and subsequently developed a lobular pneumonia which proved fatal. Additional autopsy findings consisted of acute colitis and parenchymatous degeneration of heart, liver, and kidneys. The other patient received 10 intravenous injections of gold sodium thiosulfate in the same manner, or a total of 0.95 Gm. of the drug in ten weeks. Within a few days she developed large patches resembling lichen planus that covered the arms, face, and chest. Shortly after the appearance of the skin lesions large purpuric areas appeared on the feet. Eight months after the onset of these lesions the only change seemed to be an increase in the areas of the purpura. At this time her blood platelet count was reduced to 40,000 per cubic millimeter. It would seem that the intravenous use of gold sodium thiosulfate is definitely attended with increased risk.*

PLAN OF USE

Before starting chrysotherapy the patient should have a thorough physical examination. Laboratory work should consist at least of a complete blood count, blood platelet count, red blood cell sedimentation rate, and urinalysis. As already indicated, our choice of preparations is a watery solution of gold sodium thiomalate (myochrysin). This preparation is given into the gluteal muscles at weekly intervals. The initial dose should consist of 0.01 Gm. of the salt (which contains 50 per cent gold), followed by 0.025 Gm., 0.05 Gm., and 0.1 Gm. thereafter until a total of 1.0 Gm. is given. The laboratory determinations mentioned above must be repeated at intervals not exceeding four weeks. With the appearance of any significant change (except in red blood cell sedimentation rate) the determinations should be done weekly and the gold stopped if an unsatisfactory trend appears. Frequently a return of the red blood cell sedimentation rate toward normal coincides with the clinical improvement of the patient. At other times a patient may feel much better, but the red blood cell sedimentation rate will not change. At the end of a course the patient is given a rest period of six to eight weeks. If there has been no improvement in the condition, a second, and even a third, course should be given, observing the usual precautions. Should there be marked improvement by the end of the first or second course, it is generally advisable for the patient to receive another full course after a proper rest interval.

*In a subsequent series of cases where the maximum single dose of gold sodium thiosulfate was 0.05 Gm., excellent clinical results were obtained with a minimum of minor toxic reactions.

SUMMARY

In gold salts we have a valuable drug for the treatment of rheumatoid arthritis. The use of this preparation is attended with certain risks, and the physician must be able to recognize the first manifestation of any toxic reaction. These reactions, which may occur as exacerbation of joint symptoms or as lesions of the skin, mouth, gastrointestinal tract, liver, kidneys, vasomotor apparatus, or hematopoietic system, have been described. Fatalities have been reported with the use of gold compounds subsequent to the development of exfoliative dermatitis, acute yellow atrophy of the liver, and the blood dyscrasias. It is not possible to predict the time of occurrence of reactions since they may appear any time between the first injection and a time several months after the cessation of chrysotherapy. Suggested methods of preventing and controlling reactions have not proved successful. Chrysotherapy is contraindicated in patients with involvement of liver or kidneys or the hematopoietic system and should be used with caution in those having a history of allergy. Treatment should be discontinued at the first sign of any toxic reaction and may be resumed when the signs have cleared, except in those patients who develop exfoliative dermatitis, blood dyscrasias, or severe hepatic or renal lesions.

The preparation we prefer is a watery solution of gold sodium thiomalate (myochrysin) given intragluteally in doses no larger than 0.1 Gm. at weekly intervals until a total of 1.0 Gm. has been administered. Following a rest period of six to eight weeks a second, or even a third, course is given as indicated. To properly safeguard the patient, it is essential that in addition to a thorough physical examination certain laboratory procedures be done before starting gold salt therapy. These determinations should consist of a complete blood count, blood platelet count, red blood cell sedimentation time, and urinalysis. This laboratory work should be repeated at least every four weeks, and with the appearance of any unfavorable change the determinations should be done weekly. Treatment should be discontinued if an unsatisfactory trend appears.

REFERENCES

1. Hench, P. S., Bauer, W., Dawson, M. H., Hall, F., Holbrook, W. P., and Key, J. A.: The Problem of Rheumatism and Arthritis; Review of American and English Literature for 1937 (Fifth Rheumatism Review), *Ann. Int. Med.* 12: 1005, 1939.
2. Phillips, R. T.: The Treatment of Arthritis With Gold Salts, *New Eng. J. Med.* 214: 114, 1936.
3. Oren, Hyman: Arthritis and Its Treatment With Gold Salts, *J. Med. Soc. N. J.* 33: 591, 1936.
4. Key, J. A., Rosenfeld, H., and Tjoflat, O. E.: Gold Therapy in Proliferative (Especially Atrophic) Arthritis, *J. Bone & Joint Surg.* 21: 339, 1939.
5. Sashin, D., Spanbock, J., and Kling, D. H.: Gold Therapy in Rheumatoid Arthritis, *J. Bone & Joint Surg.* 21: 723, 1939.
6. Snyder, R. G., Traeger, C., and Kelly, L.: Gold Therapy in Arthritis: Observations on 100 Cases Treated With Gold Sodium Thiosulphate and Auroccin, *Ann. Int. Med.* 12: 1672, 1939.
7. Hartfall, S. J., Garland, H. G., and Goldie, W.: Gold Treatment of Arthritis: A Review of 900 Cases, *Lancet* 2: 784, 1937.
8. Crosby, C. J. V.: The "Accidents" of Gold Treatment in Rheumatoid Arthritis, *Lancet* 1: 1463, 1936.
9. Reznikoff, Paul: Personal communication.
10. Hartfall, S. J., and Garland, H. G.: Gold Treatment of Rheumatoid Arthritis, *Lancet* 2: 8, 1935.

CLINICAL CHEMISTRY

THE CHEMISTRY OF INFECTIOUS DISEASES*

II. CYSTINE CONTENT OF THE HYDROLYSATES OF BLOOD SERA IN EXPERIMENTAL PNEUMONIA IN DOGS

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BRDICKA¹ interpreted the decrease in the height of the polarographic waves of cancerous sera as being due to less cystine, i.e., cystine plus cysteine. Applying the Heyrovsky polarograph to the study of whole sera of type I pneumococcus infected dogs, we observed that the height of the catalytic protein wave also decreased with an increase in the severity of infection, and returned to normal upon recovery of the animal.² Brdicka¹ believed that the height of the polarographic double wave of serum in cobaltic ammonium buffer is a measure of the amounts of available sulfhydryl and disulfide groups of the serum proteins, while nonproteic sulfhydryl and disulfide groups (i.e., free cysteine and cystine) in trivalent cobalt buffer are without influence² on the height of the wave. Consequently, a decrease in the wave height of whole serum with pneumococcal infection would indicate a diminution of peptide cystine. To ascertain the correctness of this deduction, the cystine content of the acid hydrolysates of dog sera before and after pneumococcal infection was determined.

EXPERIMENTAL

The care of the dogs and the production of experimental pneumonia according to the procedure of Terrell, Robertson, and Coggeshall^{3†} were described in a previous report.²

Samples of 0.4 ml. of serum were refluxed with 2.5 ml. of the formic acid-hydrochloric acid mixture of Miller and du Vigneand⁴ in an all-glass apparatus in an oil bath at 110 to 120° C. for eighteen hours. After dilution with 5 N hydrochloric acid to 10 ml., cystine was determined on 1 ml. aliquots in triplicate by the method of Vassel,⁵ care being taken to prepare the standard cystine curve from a cystine solution of identical formic acid and hydrochloric acid composition and of the same normality (average 7 N) as that of the hydrolysates. For the polarographic analyses 1 ml. of the hydrolysate was diluted with water to

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†Culture furnished through the courtesy of Dr. O. H. Robertson, of the University of Chicago.

4 ml., and 0.5 ml. of this solution was added to a cobaltous ammonium buffer of the following composition:

To 2 ml. of an 0.008 N cobaltous chloride solution was added 1 ml. of 1 N ammonium chloride, followed by 1 ml. of 1 N ammonium hydroxide and 5.5 ml. of water. The mixture was then electrolyzed with the galvanometer set at $\frac{1}{100}$ sensitivity.

The polarographic cystine values were at first determined by the procedure of Stern, Beach, and Maey.⁶ This led to results which were found to be uniformly 15 to 25 per cent lower than those obtained by the colorimetric method. When a standard curve was prepared by plotting the increase in wave height of the catalytic wave against increasing amounts of pure cystine in acid solutions of identical composition and normality with those of the hydrolysates, a calibration curve was obtained which when used on the serum hydrolysates gave cystine values which were within ± 5 per cent numerically the same as those

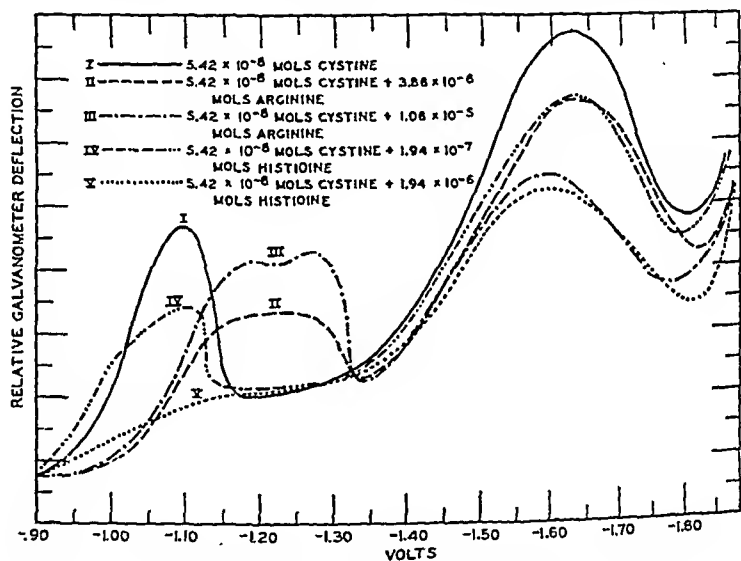


Fig. 1.—The depressing effect of arginine and histidine on the polarographic wave height of cystine in cobaltous ammonia buffer.

obtained by the procedure of Stern and others,⁶ and which were uniformly lower than those obtained by colorimetry. However, since the solution used in preparing the calibration curves contained no other amino acids than cystine, this agreement seemed surprising, for Sládek and Lipschuetz⁷ state that the presence of arginine and histidine depress the wave height of cystine polarograms. We found that the addition of these and other amino acids in amounts similar to those found in serum⁸ did not depress the wave height when added separately or together to 0.015 mg. of cystine. The following amino acids were tested in this manner: 0.17 mg. of arginine, 0.004 mg. of histidine, 0.02 mg. of tyrosine, 0.004 mg. of tryptophane, and 0.027 mg. of glutamic acid. If the amounts of arginine and histidine are increased, however, then both amino acids depress the wave height, histidine exerting a greater effect, mol for mol, than arginine. This is shown in Fig. 1, where curve I is the polarogram of 5.42×10^{-5}

mols of cystine in cobaltous ammonium buffer. The addition of 3.86×10^{-6} mols of arginine (curve II) or 0.194×10^{-6} mols of histidine (curve IV) will depress the current-voltage wave of 5.42×10^{-8} mols of cystine by 18 per cent, while 10.6×10^{-6} mols of arginine depress it 39 per cent (curve III) and 1.94×10^{-6} mols of histidine 43.5 per cent (curve V). The presence of appreciable amounts of histamine will also influence the cystine wave height. Histamine in cobaltous ammonium chloride buffer shows a maximum of its own at -1.75 volts. Since the maximum produced by cystine occurs at -1.65 volts, the presence of histamine increases the wave height of the cystine polarogram.

The procedure of Stern and associates⁶ is much more time-consuming than the use of a standard cystine curve without, apparently, gaining in accuracy. The majority of the cystine values reported here were, therefore, calculated from a cystine calibration curve, i.e., the wave height produced by pure cystine solutions were compared to that given by the hydrolysate.

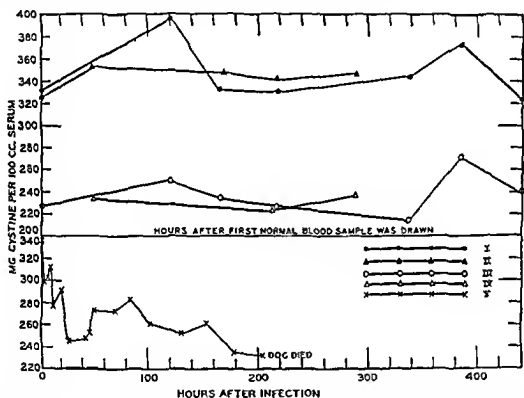


Fig. 2.—Curves I to IV. Cystine content of the serum of normal Dog XI two hours after a meal (I—colorimetric, III—polarographic) and twenty hours after a meal (II—colorimetric, IV—polarographic). Curve V—variations in cystine content of Dog XII after infection with type I pneumococcus.

RESULTS AND CONCLUSIONS

The normal variations in the cystine content of serum hydrolysates of the same dog are plotted in Fig. 2, where curves I and III represent colorimetric and polarographic cystine determinations of the blood of the same dog drawn on different days after about twenty hours of fasting. Curves II and VI represent the corresponding cystine curves two hours after feeding. Although the colorimetric and polarographic determinations vary in absolute values, the graphs of the analyses by the two methods run fairly parallel to each other. They also show that the tendency to fluctuate is independent of the time of day at which the blood was drawn and of whether or not the animal had been given food recently. These normal variations were of the same magnitude as those previ-

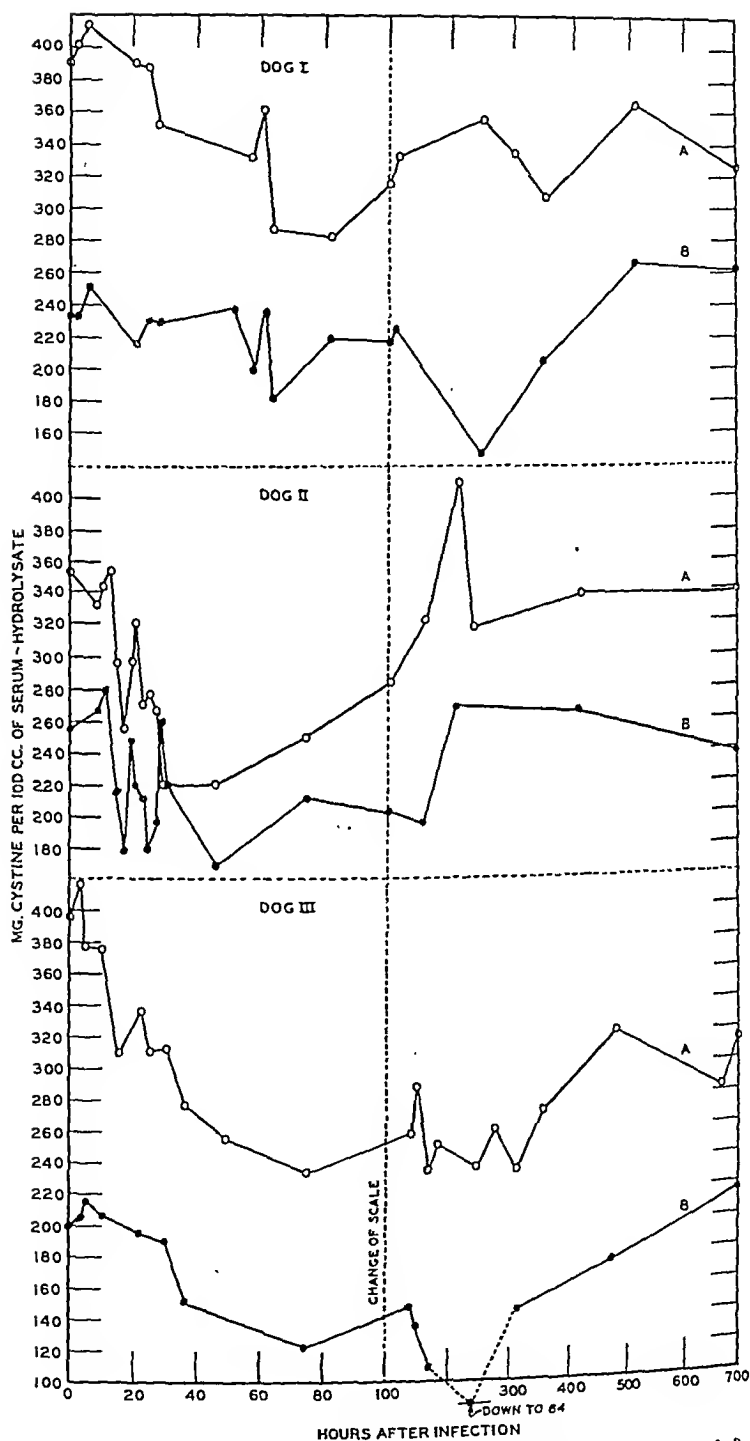


Fig. 3.—Variations in the cystine content of the serum hydrolysates of 3 dogs after infection with type I pneumococcus. Curve A—analysis by a colorimetric method; curve B—analysis by the polarographic method.

ously observed when the fluctuations of the whole sera of normal dogs were analyzed polarographically in cobaltic ammonium chloride buffer.² The lowest normal cystine value of the eight dogs is 300 and 200 mg. per cent for the colorimetric and polarographic methods, respectively, while maximum values for the two procedures were 420 and 260 mg. per cent. The maximum fluctuation of an individual dog amounted to about ± 15 per cent of the average normal value in either of the two methods, this tendency being directed more toward higher than toward lower values. It was noticed, furthermore, that the serum hydrolysates from normal puppies always contained appreciably less cystine per 100 ml. of serum than those of older animals.

During the first twenty to sixty hours after infection with type I pneumococcus, corresponding to the spreading of the infection in the lung as shown by x-ray, a sharp drop of 15 to 30 per cent of the former average cystine value of the dog serum occurred. The sudden decrease was not continuous but fluctuated, showing a strong downward tendency, this being opposite to the normal tendency.

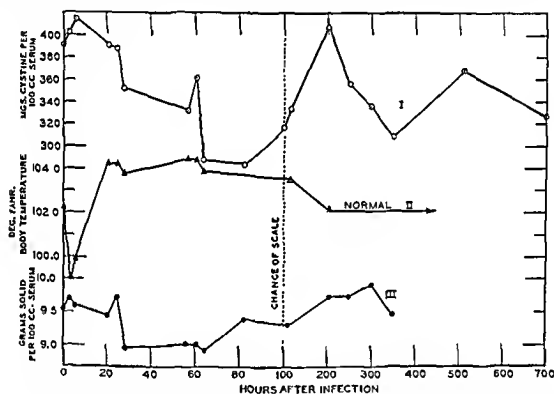


Fig 1.—Variations in cystine content (I), body temperature (II), and total solids (III) of the serum of Dog 1 after infection with type I pneumococcus.

In Fig. 3 serum cystine concentrations were plotted against time after infection for three representative cases of the eight dogs studied. The results of the colorimetric analyses are designated curves A, while curves B represent always the corresponding polarographic values. It can be seen that after the lowest concentrations were reached, the cystine values returned during the next one hundred to two hundred hours after infection to their previous normal levels, even exceeding them at times, as if by a compensatory mechanism. During this period the x-rays show that resolution of the infected area of the lungs is taking place.

From the three hundredth to the six hundredth hour after infection, corresponding to the time of recovery as measured by the polarographic peptone wave³ and by the completed resolution of the lungs, the cystine content of the serum returned to its normal level. The graph of Dog XII is of special interest,

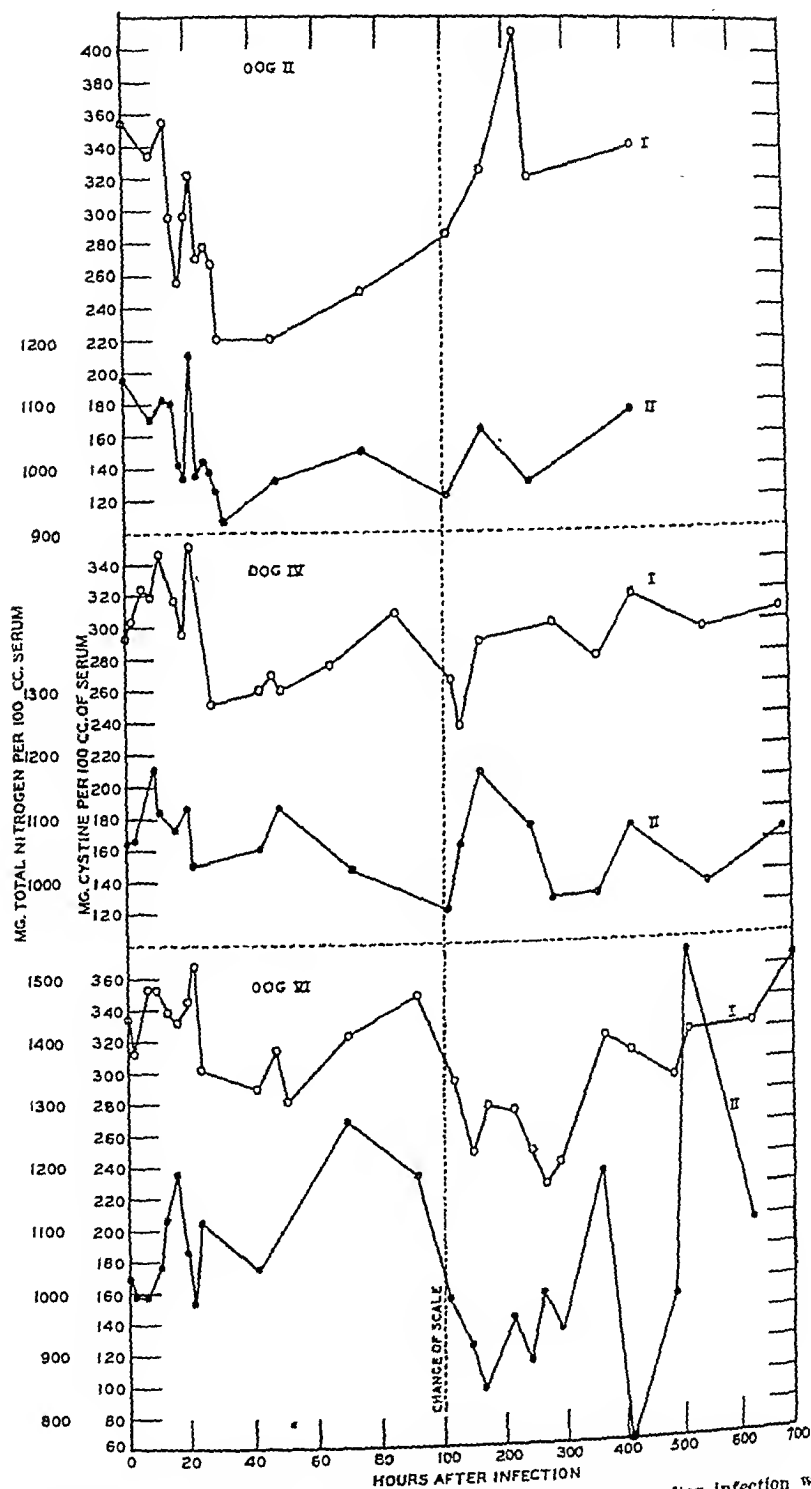


Fig. 5.—Cystine (I) and nitrogen (II) content of the sera of 3 dogs after infection with type I pneumococcus.

inasmuch as this animal died at the two hundred and fiftieth hour after infection (Fig. 2, curve V). A rather steady decrease from a normal level of 340 mg. per cent to a low of 234 mg. per 100 ml. of serum occurred during the illness, the latter value being one of the lowest cystine levels found (colorimetrically) in this study.

A comparison of the cystine concentrations of the serum hydrolysates during infection (Fig. 4, curve I) with the body temperature (Fig. 4, curve II) and with the solid contents of the serum of the same dog (Fig. 4, curve III) is very interesting. While the parallelism is not absolute, there is a tendency for the cystine level to decrease with a fall in total solids, preceded by an increase in body temperature. The first indication of pathologic symptoms is always reflected first in changes of the body temperature, while the changes in total solid and cystine values lag by seven to twenty-four hours. The low body temperatures observed during the first ten hours after infection are produced by the injection of morphine sulfate which is used to lower the resistance of the body to the invasion of the pneumococci. The drop in the serum solids reaches a maximum of 8 to 10 per cent below the normal level, while the maximum decrease in cystine concentration is from 20 to 30 per cent. In an attempt to explain this observation the total and nonprotein nitrogen values were studied in a few of the dogs. The latter decreased during infection from 50-55 to 30-45 mg. per cent, while the former fell from a normal level of 1,000-1,150 to 800-950 mg. per cent. In Fig. 5 the decrease in total nitrogen, as well as the corresponding decrease in cystine, is plotted against time after infection for Dogs I, IV, and VI. It is evident that the nitrogen and cystine curves run fairly parallel to each other. Occasional sudden and large deviations from this parallelism, especially noticeable in the case of Dog VI, cannot be explained. The maximum decrease in nitrogen amounts to 20-25 per cent, which approximates very closely the percentage drop in cystine.

Thus, during infection the observed fall in the cystine values of the serum hydrolysates are closely related to the corresponding lowering of the total nitrogen of the serum, undoubtedly representing changes in serum protein concentration. The previously observed fall in the height of the polarograms of whole dog sera with pneumococcal infection² must, therefore, be attributed also to a decrease in proteic cystine.

SUMMARY

The cystine content of the hydrolysates of dog sera after infection with type I pneumococcus was determined. Parallel analyses were made with colorimetric and polarographic analytical methods.

The colorimetric procedure gave uniformly higher values than the polarographic procedure.

Both methods showed that the cystine content of the serum hydrolysates decreased during infection, and returned to normal upon recovery.

The percentage decrease in cystine concentration of the serum hydrolysates was paralleled by the percentage fall in their total nitrogen content. This suggested that a fall in the protein level occurred during infection.

We gratefully acknowledge the invaluable help and the unstinted cooperation which we received in this study from Dr. W. Harry Feinstone of this laboratory. Dr. Feinstone, while actively studying related phases of pneumococcal infection in dogs, kindly took over the culturing of the pneumococci and the actual infection of the dogs, and followed the progress of the disease systematically with x-ray photographs.

REFERENCES

1. Brdicka, R.: Die Anwendung der polarographischen Methode Professor Heyrovsky's für die serologische Diagnostik des Krebses, *Revue des Gesundheitswesens* 20: 1, 1933.
2. Crossley, M. L., Kienle, R. H., Vassel, B., and Christopher, G. L.: The Chemistry of Infectious Diseases. I. Polarographic Study of Blood Sera in Experimental Pneumonia in Dogs, *J. LAB. & CLIN. MED.* 28: 1500, 1941.
3. Terrell, E. E., Robertson, O. H., and Coggeshall, L. T.: Experimental Pneumococcus Lobar Pneumonia in the Dog. I. Method of Production and Course of the Disease, *J. Clin. Investigation* 12: 393, 1933.
4. Miller, G. L., and du Vigneaud, V.: The Cystine Content of Insulin, *J. Biol. Chem.* 118: 101, 1937.
5. Vassel, B.: A Colorimetric Micro Method for the Estimation of Cystine and Cysteine, *J. Biol. Chem.* In Press.
6. Stern, A., Beach, E. F., and Macy, I. G.: Polarographic Microdetermination of Cystine in Protein Hydrolysates, *J. Biol. Chem.* 130: 733, 1939.
7. Sládek, J., and Lipschuetz, M.: Polarographic Studies With the Dropping Mercury Cathode. XLIII. Specific Effects of Some Amino Acids, *Collect. Czechoslov. Chem. Communicat.* 6: 487, 1934.
8. Schmidt, C. L. A.: The Chemistry of the Amino Acids and Proteins, Springfield and Baltimore, 1938, Charles C Thomas, p. 217.

EFFECT OF DESOXYCORTICOSTERONE ACETATE ON HYPOCHLOREMIA IN PNEUMONIA*

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THE present study was undertaken to determine what effect, if any, desoxycorticosterone acetate has upon the hypochloremia so frequently found in patients with pneumonia.

McLean¹ in 1915 observed that chloride is treated by the kidneys as a threshold substance and that the threshold is decreased in pneumonia. Following crisis, the threshold rises and chlorides are retained until normal serum levels are reached. The presence of a lowered renal threshold hardly provides a complete explanation for the hypochloremia; however, since desoxycorticosterone acetate, among its other effects, produces a rise in the renal threshold for chloride in Addison's disease, it was thought possible that this would result in a rise in the chloride, which might be beneficial in patients with pneumonia.

MATERIAL

For the purpose of the experiment there were selected 4 patients acutely ill with lobar pneumonia of two to five days' duration who had serum chloride concentrations of 95.1 milliequivalents per liter, or less. Detailed balance

*From the Department of Chemistry, New York University College of Medicine.
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studies were not made. The effect of desoxyeorticosterone acetate was determined by following the chloride and carbon dioxide contents of the serum. All patients were given 6 Gm. daily of sodium chloride (Lilly's Enseals). The diets were liquid or soft, without added salt except that taken as desired by the patients from weighed salt shakers. Chlorides were determined by the method of Patterson,² and the carbon dioxide content by the method of Van Slyke and Neill.³

The dosage of desoxyeorticosterone used was between 10 and 20 mg. per day (in the form of a solution in sesame oil, given intramuscularly). The results of treatment in the 4 patients are summarized in Table I.

TABLE I

CASE	DAY	NaCl INTAKE* (GM.)	DOCA† (MG.)	SERUM CHLORIDE MEQ./L.	CL + CO ₂ MEQ./L.
1	1	6.0	10	94	121
	2	6.0	10	89	121
	3	6.0	10‡	91	122
	4		0	95	
2	1	4.0	10	90	117
	2	6.2	10	90	117
	3	15.2	10	89	119
	4	15.0	0		
	5		0	92	124
3	1	7.3	20	95	122
	2	6.7	15	102	131
	3		0	104	131
4	1	6.0	0	94	123
	2	6.0	0	100	132
	3		0	104	132

*In addition to amount in "salt-free" diet.

†Desoxyeorticosterone acetate.

‡Plus 10 c.c. Cortin (Upjohn) intravenously.

RESULTS

Daily 10 mg. doses of desoxyeorticosterone acetate did not appear to raise significantly either the serum chloride level or the sum of the carbon dioxide and chloride levels in pneumonia patients who were given not less than 6 Gm. of salt a day. In Case 3 where larger doses of the hormone were given, there was a moderate and steady rise in the serum chlorides, but as striking a rise took place in Case 4 where no desoxyeorticosterone acetate was given.

Clinically, there was no difference to be noted between patients receiving desoxyeorticosterone acetate and those who did not, nor did the chloride levels, either initially or after treatment, seem to parallel in any way the clinical state of the patients. All 4 patients were under treatment with sulfonamide derivatives.

These findings are similar to those reported by Winkler and Crankshaw⁴ who were unable to raise the serum chloride and sodium concentrations substantially by the administration of salt and potent cortical hormone in a number of patients with hypochloremia accompanying tuberculosis, carcinoma of the lung, and arteriosclerotic heart disease.

SUMMARY AND CONCLUSIONS

Of 4 patients with lobar pneumonia who had low serum chloride levels, 3 were treated with desoxycorticosterone acetate, while one received salt alone. Ten milligrams daily of desoxycorticosterone acetate had no effect, and 15 to 20 mg. had a very doubtful effect upon the serum chloride. Clinically, there seemed to be no benefit to pneumonia patients treated with desoxycorticosterone acetate in the dosages administered.

REFERENCES

1. McLean, F. C.: The Numerical Laws Governing the Excretion of Urea and Chlorides in Man. I. An Index of Urea Excretion and the Normal Excretion of Urea and Chlorides, *J. Exper. Med.* 22: 212, 1915. II. Influence of Pathological Conditions and of Drugs on Excretion, *J. Exper. Med.* 22: 366, 1915.
2. Peters, J. P., and Van Slyke, D. D.: Quantitative Clinical Chemistry, Baltimore, 1932, Williams & Wilkins Co., Vol. II (Methods), p. 838.
3. Same, p. 285.
4. Winkler, A. W., and Crankshaw, O. F.: Chloride Depletion in Conditions Other Than Addison's Disease, *J. Clin. Investigation* 17: 1, 1938.

BLOOD SERUM CALCIUM IN THE NEGRO*

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THE paucity of data in the literature on the serum calcium content of the blood of Negroes in the United States and environs constitutes a gap in our knowledge which bridged might be of value in explaining racial differences in susceptibility and response to treatment in certain diseases. The following report represents a study designed to establish a normal value for blood calcium in this group.

EXPERIMENTAL

A total of 385 determinations on blood serum calcium of 309 normal persons were carried out. All of them were American or foreign-born Negroes, representing thirty-two states, the District of Columbia, the Virgin Islands, and four foreign countries. The subjects were students, professors, instructors, technicians, secretaries, and janitors from the medical school, the George W. Hubbard Hospital, and a group of subjects including six children from a WPA Youth Center in Nashville.

Blood was collected in the postabsorptive state. Samples were received in 15 c.c. centrifuge tubes with careful handling in order that the erythrocytes might not be injured and a hemolytic serum be produced. The blood was allowed to clot, and the material was subjected to centrifugation for more com-

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plete separation of the serum and the clot. Tubes were stoppered during centrifugation—loss of water through evaporation being sufficiently appreciable to give high values for the calcium.

Analyses were carried out according to the Clark-Collip modification of the Kramer-Tisdall procedure,² and all determinations were done in duplicate. If any red blood cells or hemoglobin were present following precipitation of

TABLE I
BLOOD SERUM CALCIUM IN NORMAL SUBJECTS

SUBJECTS	NUMBER	RANGE	MEAN	STANDARD DEVIATION
Normals	385	9.03-11.86	10.39	0.65
Male				
Adults	341	9.03-11.86	10.41	0.69
Female				
Adults	38	9.53-11.05	10.24	0.46
Children	6	9.69-11.42	10.30	0.57

calcium as the oxalate, they were removed as completely as possible in the subsequent washings in order that absence of any reducing noncalcium oxalate substances might be assured. The permanganate titration was carried out in tubes held in a water bath at 70° to 75° C. The criterion for the normal state was based upon the results of a thorough physical examination, including serologic tests executed by a corps of technicians from the medical and hospital staff.

Serum calcium was determined twice on blood from 76 normal subjects—the second samples being taken after an interval of from seven to twelve months.

RESULTS

The results of the experiment are shown in Table I.

DISCUSSION

Unique in the literature on the subject of blood serum calcium in Negroes is a paper¹ in which the conclusion is reached that serum calcium averages 10.93 mg. per 100 c.c.*—a value 0.43 mg. per 100 c.c. higher than the normal blood serum calcium for white persons which is given as 10.50 mg. per 100 c.c. This normal was established on the basis of an average of forty-four determinations. Neither the range of values obtained nor the method used was given. Differences in susceptibility to, and treatment of, tuberculosis in Negroes were explained in part on this basis.

In the present work a larger series was used and the subjects were taken from widely separated areas. The majority of the subjects were students and blood samples were taken shortly after their arrival in Nashville. Values obtained fell within the range of 9.03 to 11.86 mg. per 100 c.c., with a mean of 10.39 mg. per 100 c.c. Since the normal value for blood serum calcium varies from 9.00 to 11.50 mg. per 100 c.c.,³ with a mean value of 10.3,³ it would seem

*In the article cited the values actually given were expressed in grams per 100 c.c. The author could not be reached but it was assumed that these were intended to read milligrams per 100 c.c.

on the basis of the values obtained by us that the blood serum calcium for the group here investigated is of the same order of magnitude as is that of others.

Of interest in this connection are the reports of Radna⁴ who found blood serum calcium in 134 natives of the Belgian Congo to vary from 9.97 to 10.73 mg. per 100 c.c., and of Stone,⁵ who suggested that the normal European range (9.00 to 11.00 mg. per 100 c.c.) for calcium should be adhered to in the interpretation of calcium values of normal Rhodesian natives.

Thanks are due to Mr. T. A. Jones for technical assistance, and to Dr. F. W. Claytor and Dr. J. S. Chandler for obtaining the blood samples. Above all are we grateful to the Dean of the College, Dr. E. L. Turner, without whose interest and cooperation this work would not have been possible.

CONCLUSIONS

1. A total of 385 determinations of blood serum calcium on 309 Negroes were carried out.
2. Blood serum calcium in this group is of the same order of magnitude as is found in other groups of the population.
3. These results are not in accord with those of Harrell.¹
4. Supposed racial differences in susceptibility and response to treatment in tuberculosis cannot be predicated on differences in blood serum calcium.

REFERENCES

1. Harrell, C. L.: Blood-Serum Calcium in the Negro, *Am. Rev. Tuberc.* 19: 350, 1929.
2. Clark, E. P., and Collip, J. B.: A Study of the Tisdall Method for the Determination of Blood Serum Calcium With a Suggested Modification, *J. Biol. Chem.* 63: 461, 1925.
Tisdall, F. F.: A Note on the Kramer-Tisdall Method for the Determination of Calcium in Small Amounts of Serum, *J. Biol. Chem.* 56: 439, 1923.
Kramer, B., and Tisdall, F. F.: A Simple Technique for the Determination of Calcium and Magnesium in Small Amounts of Serum, *J. Biol. Chem.* 47: 475, 1921.
Clark, G. W.: The Micro Determination of Calcium in Whole Blood, Plasma and Serum by Direct Precipitation, *J. Biol. Chem.* 49: 487, 1921.
3. Schmidt, C. L. A., and Greenberg, D. M.: Occurrence, Transport and Regulation of Calcium, Magnesium and Phosphorus in the Animal Organism, *Physiol. Rev.* 15: 297, 1935.
4. Radna, R.: Sur la teneur en Calcium du sérum des indigènes du Congo Belge, *Ann. Soc. belge de med. trop.* 19: 61, 1939.
5. Stone, W.: The Blood Chemistry of Normal Southern Rhodesian Natives, *Tr. Roy. Soc. Trop. Med. & Hyg.* 30: 165, 1936.

LABORATORY METHODS

GENERAL

A SIMPLE CONSTANT INJECTION APPARATUS*

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IN OUR laboratory we have developed a practical and efficient apparatus for constant injection which has such advantages that we want to make its details known for the use of other investigators.

Two types of constant injection pumps are most frequently used. First, a syringe, the barrel of which is moved forward mechanically,¹ and second, a pump with valves, by means of which the fluid from a container is aspirated and ejected into the animal. The best known pump of this type is the Woodyatt pump,² and it is a modification of this apparatus that we are reporting here. The rotor type of injection pump avoids the use of valves but is better suited for injection of larger amounts of fluid.

Description of Apparatus.—A 110 volt, 2 amperes, $\frac{1}{20}$ horse power, 60 cycle constant speed motor with 1,125 r.p.m. is employed.† The motor has a built-in reduction gear with a ratio of 40:1. The gear of the motor is connected to a reduction gear box, as shown in Fig. 1. A simple gear shift (*A*), as used by Soskin in the most recent modification of his constant injection pump,¹ permits easy shifting between the seven variable speeds. The transmitting axis of the gear box is connected to a stroke length adjuster which is manipulated by a screw (*B*) and can be set by a wing screw (*C*). The rotating motion of the stroke length adjuster is transformed to horizontal linear motion by *D*, which is

TABLE I

VOLUME EJECTED BY PUMP AT STROKE LENGTH OF 10 MM. AND VARYING SPEEDS

TIME IN MIN.	VOLUME IN C.C.						
	1ST SPEED	2ND SPEED	3RD SPEED	4TH SPEED	5TH SPEED	6TH SPEED	7TH SPEED
	1½ S.P.M.*	3 S.P.M.	6 S.P.M.	12 S.P.M.	24 S.P.M.	48 S.P.M.	96 S.P.M.
15	8	15.5	30	59	116	235	460
30	15.5	30.2	60	118	232	465	920
45	23.5	45.5	90	176	350	700	1,380
60	31.0	60.5	120	235	466	930	1,840

*S.P.M. = Strokes per minute.

transmitted to the piston of a 1 c.c. tuberculin syringe by way of a screw cap attachment *E* to the top of the piston of a syringe. The tuberculin syringe is adjusted to exactly the same height as *D* in an adjustable support in which it

*From the Department of Gastro-Intestinal Research, Michael Reese Hospital, Chicago.

†Bought from the Bodine Motor Co., Chicago, Type NSA53R.

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is elamped down tightly. Larger syringes can also be used. The tip of the syringe is connected by pressure tubing to a small T-piece *F*, the ends of which are connected to the valves. All (glass) connections are glass to glass. The valves are constructed in the following manner (Fig. 2): A piece of thick capillary glass tubing is covered on one side by a thin dental rubber dam which is tied to it on a small groove. At the side of the capillary tube a small incision is made into the rubber membrane with a razor blade, and the capillary tube and the glass jacket are connected by means of a piece of heavy rubber tubing. This valve has the advantage of great simplicity, small dead space, no backflow, and practically no elastic back pressure. The intake part of the valvular system dips directly into a beaker containing the fluid to be injected. This makes it easy to change from solution to solution for injection purposes. The outlet

CONSTANT INJECTION PUMP

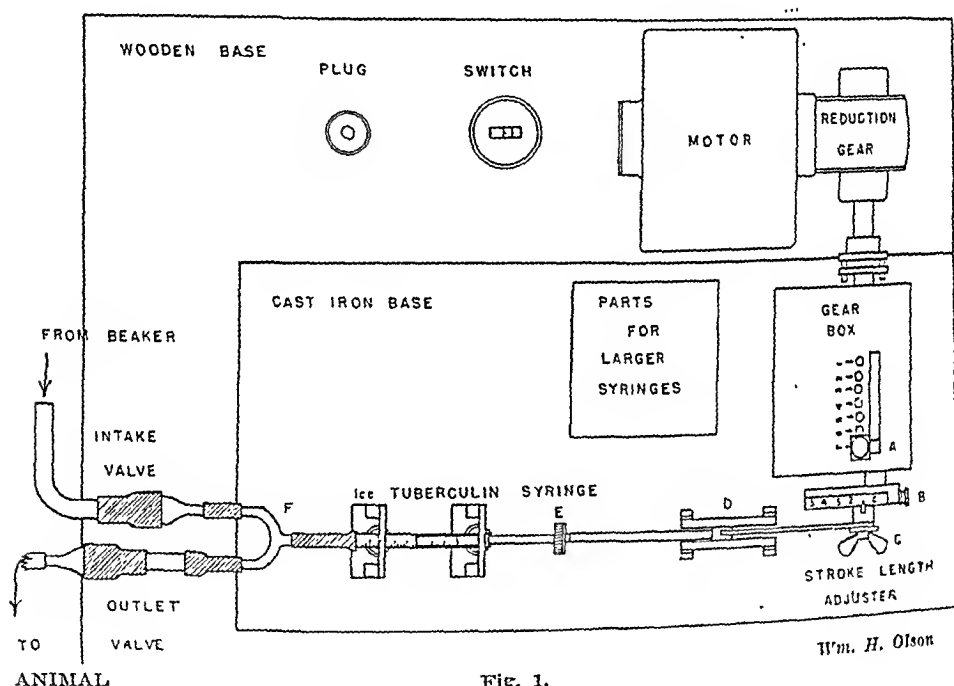


Fig. 1.

valve is connected to the injection cannula in the animal through a thin rubber tube. The volume of fluid in the complete valvular system is 6 c.c. Table I demonstrates results obtained in tests with the assembled instrument. Each number is the average of three determinations, except the last one which was repeated ten times. The tests were extended over a period of three days, with no change in the degree of accuracy of performance. In order to obtain exact results all rubber tube to glass connections must be wired, air bubbles must be eliminated, and the solutions must be gas free. The syringe must be renewed from time to time because the constant motion tends to grind down the piston. The piston must be greased with stopecock grease. As can be seen from Table I there is a nearly linear relationship between the ejected volume of fluid and the number of strokes, degree of gear reduction, and time. The small deviations from an

exact linear relationship are within the limits of error of our readings of the volumes in graduated cylinders. If one does not want to check the pump regularly and rely on previous checks, the volume of injected fluid can be read conveniently from a graduated cylinder into which the intake tube is introduced.

Injection by this pump is not continuous but rhythmic, varying between $1\frac{1}{2}$ and 96 strokes per minute. The volume varies between approximately 0.5 and 30 c.c. per minute, at a stroke length of 10 mm. By reducing the stroke length proportionately smaller amounts and by increasing it, proportionately larger amounts may be injected. The apparatus has been in use in the laboratory for more than two years and has been found satisfactory.

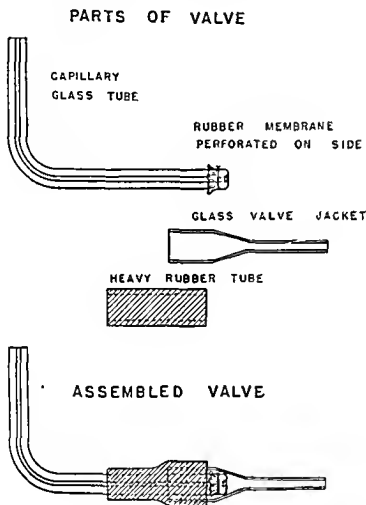


Fig. 2.

SUMMARY

A constant injection pump is described in which speed and stroke are adjustable. Simple and efficient rubber membrane valves are used which do not leak and have a minimum of elasticity to back pressure. The instrument was constructed for us by our mechanic, Mr. Carl Griesmeyer.

REFERENCES

1. Soskin, S.: A Simple Constant Injection Apparatus, *J. LAB. & CLIN. MED.* 18: 420, 1933.
2. Woodyatt, R. T.: The Method of Timed Intravenous Injections, *J. Biol. Chem.* 29: 355, 1917.

A CELLOPHANE STRAW USED AS AN INK-WRITING POINTER IN KYMOGRAPHIC WORK*

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IN STUDYING the respiratory pattern of psychiatric patients it was necessary to obtain long, continuous pneumographic tracings. Since this was impractical when carried out by means of the standard smoked-drum technique, an inexpensive ink-writing setup was devised, which not only solved the main problem mentioned above but was rugged enough to withstand sudden psychomotor fluctuations noted in state hospital patients, and, at the same time, eliminated the hazard of fire when recording was done on the regular wards. This ink-writing pneumographic system consists essentially of an ordinary cellophane straw with the tip fashioned and trimmed to resemble a small quill.

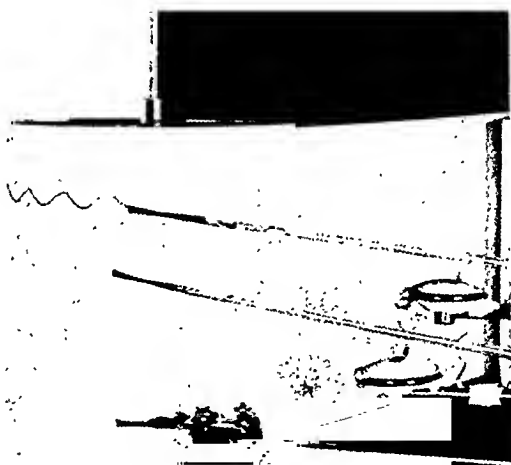


Fig. 1.

The entire apparatus is designed as follows: About 2 to 3 cm. of the end of the straw is pressed flat with the fingers and then folded in two lengthwise. A 5 mm. tip, trimmed to a sharp point, is bent at a right angle. This end is dipped into ordinary fountain pen ink to which has been added gum tragacanth

*From the Metropolitan State Hospital, Waltham.
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solution, 5 c.c. per 2 ounce bottle. The ink is drawn up by oral suction to a 2 to 3 cm. level in the straw. The other end of the straw is inserted into two celluloid "sights," one glued to the center of the tambour and one on the air pipe behind the tambour, keeping the straw straight and acting as fulcrum. A 3 to 4 cm. length of straw attached to an elongated arm of the signal magnet may serve as an ink-writing time marker. If the motor-driven Harvard kymograph is used, cheap paper may be purchased in large rolls of 6 inch width from any paper mill. This is rolled on the small drum, threaded backward around the other small drum and fastened with tape to the recording drum so that as the tracing is made the paper is automatically wound up. The ink dries by the time the tracing has gone around the drum. With this procedure, long, continuous, ink tracings may be made with the pointer moving in the vertical plane.

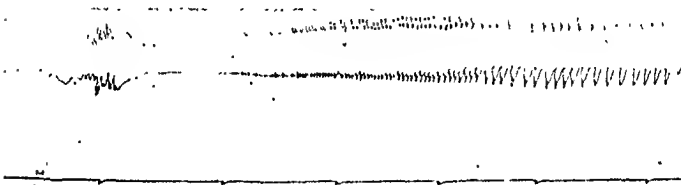


FIG. 2.

Fig. 1 illustrates the setup of the cellophane straws on a standard motor-driven Harvard kymograph.

Fig. 2 is an abdominal and thoracic pneumograph of an induced metrazol convulsion. This tracing illustrates the ruggedness and apparent accuracy of this ink-writing straw.

The setup, as described, has given satisfactory ink-written pneumographic tracings as well as records of certain muscular movements for the past year. The lightness of this cellophane straw and the simplicity of the described apparatus, with the elimination of time spent on the smoked-drum technique, should make this a usable setup, especially in student and hospital laboratories.

POSSIBLE SIGNIFICANT ERRORS IN ROUTINE TUBERCULIN TESTS*

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IN A GENERAL hospital the intracutaneous tuberculin test is widely used for diagnostic purposes. The substance usually employed is old tuberculin (O.T.), although in recent years the purified protein derivative (P.P.D.), a highly refined but more expensive tuberculo-protein, has found extensive use.

In the preparation of very dilute solutions of tuberculin for skin tests, there are possible a number of technical errors, some of which have been repeatedly pointed out in the literature. Thus, Parish and O'Brien (1935) have amply demonstrated that the usual cleaning and sterilizing procedures fail to destroy or to remove an appreciable quantity of residual tuberculin from glassware with which this highly tenacious and stable substance was previously in contact. Nelson, Seibert, and Long (1937) have reaffirmed the remarkable heat stability and adherence to glass, and have accordingly advised the use of individual, marked syringes for each dilution.

Most standard textbooks on clinical and laboratory procedure mention no detailed method for diluting old tuberculin, while often discussing minutely the actual method of performing the test itself. In those few manuals that do discuss dilution procedure, a variety of methods is described. A review of the current literature reveals a similar variation in methods suggested for the preparation of dilutions of old tuberculin.

In canvassing a number of large general hospitals, we were again struck with the variety of methods used to prepare solutions of tuberculin. This variation in dilution technique was confined to methods employing old tuberculin. Institutions using purified protein derivative, which is in the form of tablets of known weight, show more uniformity in procedure, thus reflecting the greater ease with which this substance may be handled. The following is a procedure which, according to our information, is likely to be used in some instances:

Dilutions of tuberculin are prepared by drawing up 0.1 c.c. of old tuberculin (supplied by the New York City Department of Health) in a standard 1 c.c. "tuberculin syringe" graduated in tenths, twentieths, and hundredths of a cubic centimeter. Saline is drawn up to the 1 c.c. mark. An air bubble is then introduced, and by repeated inversion, the two solutions are mixed to form a 1:10 dilution of old tuberculin. The syringe is then emptied to the point where only 0.1 c.c. remains, and by repeated manipulations as above described, the dilutions 1:100, 1:1,000 . . . 1:100,000 are prepared, all in one syringe. Intradermal testing is then carried out, still with the very same syringe, after stopping at the dilution desired. That such a procedure introduces large errors in the estimation of the degree of skin sensitivity to tuberculin seems obvious and will become more evident.

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Patients on the Medical Wards of the Mount Sinai Hospital, New York City, were subjected to comparative tests: On the skin of one forearm (flexor surface) a 0.1 c.c. intradermal wheal was raised with a "1:100,000" solution of old tuberculin prepared as above described. The other forearm was similarly and simultaneously injected with 0.1 c.c. of 1:100,000 solution of old tuberculin prepared in individual test tubes as follows:

0.5 c.c. of O.T. + 4.5 c.c. of 0.9 per cent saline solution = 1:10 dilution.

0.5 c.c. of 1:10 dilution + 4.5 c.c. of saline = 1:100 dilution.

0.5 c.c. of 1:100 dilution + 4.5 c.c. of saline = 1:1,000 dilution, etc.

A single standardized 5 c.c. pipette was used to deliver the saline, but different 1 c.c. pipettes, calibrated in tenths of a cubic centimeter (also standardized*), were used for each dilution of old tuberculin, and were marked so that they would not be used for other dilutions. The amounts of solution were relatively large and easily handled, so that errors in measuring were thereby made minimal. Tuberculin of the same lot and preparation numbers was employed for all experiments (preparation No. 142, lot No. 173). Readings were taken at twenty-four, forty-eight, and often seventy-two hours. Those at the end of forty-eight hours are listed in Table I.

From Table I it is readily seen that even a 1:10,000 dilution prepared by the "multiple pipette" method is weaker than the so-called 1:100,000 dilution of the "single syringe" procedure. (Conforming to the practice of most workers in the field, we considered size as the main indicator of the intensity of the tuberculin reaction. This is the most easily quantitated feature of the response to any given dilution, and, we believe, roughly parallels the intensity of the reaction. Our scale for classification of results is indicated in Table I.)

TABLE I

DILUTION	NO. OF CASES	"SINGLE SYRINGE" PROCEDURE					"MULTIPLE PIPETTE" METHOD				
		DIAMETER									
		<5	5-10	11-15	16-20	>20					
		mm.	mm.	mm.	mm.	mm.					
		0	1+	2+	3+	4+	0	1+	2+	3+	4+
1:100,000	33	12	0	6	4	11	30	3	0	0	0
1:10,000	14* of above cases						1†	6	2	3	2

*These tests were done seventy-two hours after the former ones, always distal to areas previously tested. All cases in this group were previously positive only to the 1:100,000 dilution prepared by the "single syringe" procedure.

†This case was then retested with a 1:1,000 dilution prepared by the "multiple pipette" method and was found positive (2+). It was previously positive (4+) to a 1:100,000 dilution prepared by the "single syringe" procedure.

Another type of comparison was attempted on a series of 9 patients who had not been previously tested. In one forearm 0.1 c.c. of a 1:1,000,000 dilution ("single syringe" procedure) was intracutaneously administered, while simultaneously, 0.1 c.c. of a 1:10,000 dilution ("multiple pipette" method) was injected into the skin of the other forearm. The results (after forty-eight hours) follow in Table II.

*One cubic centimeter pipettes ordinarily do not deliver exactly 1 c.c. of fluid. Standardized pipettes are, therefore, recommended for more accurate dilution.

TABLE II

PATIENT NO.	"SINGLE SYRINGE" PROCEDURE	"MULTIPLE PIPETTE" METHOD
	1:1,000,000 DILUTION OF O.T.	1:10,000 DILUTION OF O.T.
1	2+	1+
2	2+	1+
3	0	0
4	3+	1+
5	2+	1+
6	1+	1+
7	1+	0
8	2+	1+
9	2+	1+

It is easily seen from Table II that the "single syringe" method is abnormally sensitive. That this is due to a combination of errors will be demonstrated.

The effects of adherence to glassware on the accuracy of tuberculin dilution were briefly studied. A comparison of dilutions prepared by the "multiple pipette" method was made with a similar set of dilutions prepared by using a single pipette throughout. Arbitrarily, the pipette was rinsed five times in each successive dilution in the course of the latter method. Four patients were tested simultaneously.

TABLE III

PATIENT NO.	1:100,000 DILUTION OF O.T.		1:10,000 DILUTION OF O.T. (TESTED 72 HOURS AFTER 1:100,000 TESTS)	
	MULTIPLE PIPETTE METHOD	SINGLE PIPETTE PROCEDURE	MULTIPLE PIPETTE METHOD	SINGLE PIPETTE PROCEDURE
1	0	0	0 (2+ with 1:1,000)	3+
2	0	1+	1+	Not tested
3	0	1+	3+ (16 by 15 mm.)	3+ (20 by 18 mm.)
4	0	1+	2+ (11 by 10 mm.)	2+ (14 by 13 mm.)

Undoubtedly the reasons for the discrepancy between the multiple and single pipette methods lie in the adherence to the pipette of sufficient amount of tuberculin to increase irregularly the concentration of a given solution above its presumed potency.

It will be noted that this discrepancy is less than that observed when the "single syringe" procedure is compared with the "multiple pipette" method. This is accounted for by another source of error in the syringe method, namely, the inadvertent seepage of fluid between the barrel and piston. We attempted to estimate the extent of this error.

Slightly more than 0.1 c.c. of old tuberculin was drawn into a standard tuberculin syringe. The syringe point was then turned upwards to expel the excess solution and the few small air bubbles which are almost always drawn in, so that only 0.1 c.c. of old tuberculin finally remained. When this was done, it became evident that, by capillarity and by gravity, tuberculin had seeped between barrel and piston along the shaft of the syringe to points varying usually between the 0.40 and 0.70 c.c. marks (results of a number of tests). This type

of seepage is clearly illustrated in Fig. 1. The volume of the space between barrel and piston was then actually calculated. This was done as follows:

The diameter of the piston was easily measured to the ten-thousandth of an inch by a suitable micrometer (Starrett Micrometer No. 113). Then the diameter corresponding to that of the barrel cavity was ascertained by the use of a gradually tapering metal cone which was inserted tightly into the syringe barrel as far as it would go. The diameter of the cone at the plane of impingement against the inner wall of the syringe was measured with the micrometer. This value checked with a measurement of a paraffin cast of the barrel cavity.

From these data it is possible to calculate the actual volume of fluid that seeps for a given distance between the barrel and the normally fitting piston, by means of the formula:

$$\text{Seepage Volume} = V - v = \pi h (R^2 - r^2)$$

where V and v represent the volumes of the barrel cavity and piston, respectively, over a given length, h , with R and r representing the radii of their



Fig. 1.—Note seepage of tuberculin into space between piston and barrel

respective cross sections. Calculated to the 1 c.c. mark, the seepage space is found to equal fully 13 c.mm. or an error of about one part in 75. This introduction of more concentrated old tuberculin into successive dilutions constitutes an additional source of error in this method of dilution.

If one adds to this the "human" error which must enter into the handling of so closely graduated an instrument, the use of a syringe method of dilution becomes still further open to condemnation.

It becomes apparent, then, that a method which employs a single syringe for both dilution and skin testing is open to many errors, most of which act in one direction, namely, to make the final dilution stronger than actually was intended. These are (1) the error due to the affinity of tuberculin for glassware; (2) the error due to seepage of old tuberculin between barrel and piston; and (3) the "human" error, which is greater with a finely graduated, relatively poorly controlled instrument, such as a syringe, as contrasted with a pipette.

COMMENT

Various workers have shown that individuals with active tuberculous processes are usually far more sensitive to tuberculin skin tests than are the posi-

tive reactors in control groups. Indeed, it has been suggested by some that the test with dilute tuberculin may be of use in screening out the actively tuberculous from the general population. Thus Ayman (1937) stated that 97 per cent of clinically nontuberculous patients failed to react to a 1:50,000 dilution of old tuberculin, while 93 per cent of patients with active (nonterminal) tuberculosis reacted positively if a 9 by 10 mm. skin lesion was taken as the measure of a true positive intradermal test. (This does not quite correspond to a 2+ by our scale of measurement.) Similar findings have been reported by others (Pan, 1937 and Hunt, 1939).

The few figures which we have presented lend confirmation to this impression inasmuch as none of our ward patients, all clinically nontuberculous, gave reactions to a 1:100,000 dilution ("multiple pipette" method) measuring 9 by 10 mm. Two individuals with active tuberculosis we tested, however, did react to high dilutions; one to 1:100,000, and the second to 1:50,000. On the other hand, fully 64 per cent of our nontuberculous patients reacted to a 1:100,000 dilution prepared by the "single syringe" procedure.

It is suggested that one of two methods be employed by the general hospital in the preparation of weak solutions of tuberculin: (1) that purified protein derivative be used. This is a substance of standard purity and potency which is relatively easy to handle with a minimum of error; or (2) that dilutions of old tuberculin be prepared in a central portion of the hospital, preferably in the bacteriologic laboratory where a proper technique may be employed. Dilute solutions* will, if sterile and preserved with 0.25 per cent phenol, remain potent for at least one week. (Long and Seibert; Lincoln, Raia, and Gilbert). The brief may be made for old tuberculin that it has a vast literature behind it.

For the actual test, different, labeled syringes should be used for each dilution, regardless of whether purified protein derivative or old tuberculin is employed.

SUMMARY

(1) The possible errors attendant in the dilution and use of old tuberculin for intradermal testing have been emphasized.

(2) The use of the "single syringe" procedure, which may give rise to an error of tenfold to hundredfold in the preparation of dilutions, is inadvisable for tuberculin as well as other biological solutions.

(3) A reasonably accurate method for preparing such weak solutions has been suggested.

(4) Confirmatory evidence as to the possible significance of skin sensitivity to high dilutions of tuberculo-protein is presented.

REFERENCES

1. Parish, H. J., and O'Brien, R. A.: Heat Stability and "Tenacity" of Tuberculin, *Brit. M. J.* 1: 1018, 1935.
2. Nelson, W. E., Seibert, F. B., and Long, E. R.: Technical Factors Affecting the Tuberculin Test, *J. A. M. A.* 108: 2179, 1937.
3. Sutherland, H.: The Tuberculin Handbook, London, 1936, Oxford University Press, p. 45.
4. Nicholson, D.: Laboratory Medicine, Philadelphia, 1937, Lea & Febiger, p. 207.
5. Pirquet, C., and Koch, H.: Abderhalden's Handb. d. biol. Arbeitsmeth. 13: 304, 1924.
6. Ayman, D.: The Intracutaneous Quantitative Tuberculin Test in the Diagnosis of Active Tuberculosis, *J. A. M. A.* 103: 154, 1937.

*We consider the "multiple pipette" method sufficiently accurate and expedient.

7. Pan, J. S.: The Intracutaneous Quantitative Tuberculin Test in Active Pulmonary Tuberculosis, *Chinese M. J.* 51: 979, 1937.
- Hunt, L. W.: Roentgenograms of the Chest and the Intracutaneous Tuberculin Test for Adults. A Comparative Study, *Arch. Int. Med.* 64: 49, 1939.
8. Long, E. R., and Seibert, F. B.: Further Studies on Purified Protein Derivative of Tuberculin (P. P. D.); Its Diagnostic Value and Keeping Qualities in Dilutions, *Am. Rev. Tuberc.* 35: 281, 1937.
9. Lincoln, E. M., Raia, A., and Gilbert, L. A.: Tuberculin Reactions in Children, *J. Pediat.* 12: 479, 1938.

STAND FOR ASEPTIC OPERATIONS WITH HORSLEY-CLARKE'S STEREOTAXIC INSTRUMENT*

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THE placing of cerebral lesions by means of the Horsley-Clarke¹ stereotaxic apparatus under sterile conditions can be facilitated considerably by employing the stand described below.

A base plate fixed to the animal board by clamps carries on each side an anterior column *A* (Fig. 1) for fixation of the orbits, and a posterior column *P* for fixation of the external auditory canals. *P* carries the aural pivot *p*, which is inserted in the funnel of the ear plug; *p* is fixed by a screw 1. *P* also carries a slot *g* into which the lateral bar of the horizontal frame of the stereotaxic apparatus is inserted. The screw 2 holds the frame in place.

The column *A* carries the following parts: (1) the infraorbital brackets *b* which rest upon the lower margin of the orbit; the brackets can be moved in the horizontal plane toward or away from the midline, and forward and backward, and are held in place by the screws 3 and 4. Thus the orbital and nasal plates of the Horsley-Clarke apparatus are not needed. (2) A horizontal bar *B* with posts *h* upon which the head holder rests. The bar *B* can be moved vertically in the slot *sl*, so that the upper jaws of the animal are pressed against the brackets *b*. (3) A groove *G* into which small horizontal bars fit that are fixed on the lateral bar of the horizontal frame of the stereotaxic apparatus. The screw 5, which can be rotated in the frontal plane, lies horizontally (as shown in the figure) when it is not used, and stands vertically when it holds the apparatus in place.

The axis of the aural pivots *p* and the undersurface of the infraorbital brackets *b* lie on a plane parallel to the base plate. The bottoms of the grooves *g* and *G* that receive the stereotaxic apparatus lie in a second horizontal plane so that the base frame of the stereotaxic apparatus is parallel to the plane determined by the external meatus and the lower margin of the orbits. The median sagittal plane is indicated by a small vertical plate *pl*.

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The operation is performed in the following steps: The head of the animal (cat) is placed in a Czermak head holder (of the Harvard Instrument Company); the ear plugs and the aural pivots are inserted; the brackets *b* are placed on the lower margin of the orbits, and the upper jaws are pressed against

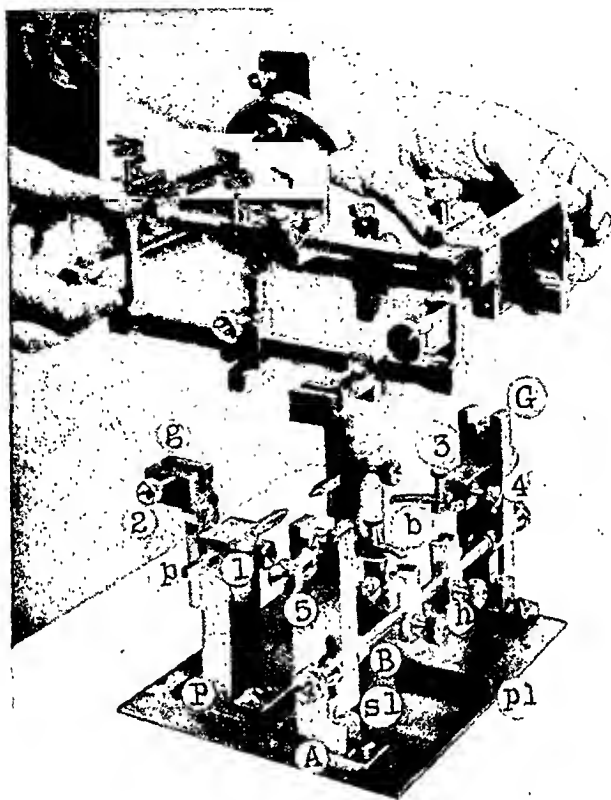


Fig. 1.—Stereotaxic apparatus held above the stand. *A*, anterior column with orbital brackets *b*, groove *G*, and bar *B* with posts *h* for head holder. *P*, Posterior column with aural pivot *p* and groove *G*. 1-5: screws; *pl* indicates the median sagittal plane. The needle of the stereotaxic apparatus is omitted.

the brackets by raising the bar upon which the head holder rests. The head is shaved and the brain is aseptically exposed. The stereotaxic apparatus is then placed on the frame and fixed by screws 2 and 5. Upon completion of the operation the stereotaxic apparatus is removed and the wound is closed.

REFERENCE

1. Horsley, V., and Clarke, R. H.: *Brain* 31: 45, 1908.

The apparatus was built by Mr. J. Davies, machine shop of Temple University School of Medicine.

TECHNIQUES FOR OBTAINING ELECTROENCEPHALOGRAMS USING OCULAR AND INTRANASAL ELECTRODES*

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ELECTROENCEPHALOGRAPHIC procedures have now come into general use for the localization of certain types of brain pathology, such as tumors and epileptic foci.¹ For this purpose, it is desirable to take off potentials at points as close as possible to the pathologic locus. Up to the present time exploration has been limited to points on the outer surface of the cranial vault. The eye being, anatomically, part of the brain, it was thought to be of interest to develop a method for studying potentials taken directly from the surface of this organ. With an electrode applied to the cornea, the soft tissues within the orbit, including the eyeball, would behave, electrically, like a mass of absorbent cotton soaked in normal saline. Such a diffuse electrode would be expected to take off potentials (1) indirectly from the frontal lobe through the orbital roof; and (2) directly from the region of the chiasma and the hypothalamus via the optic nerve. An ocular electrode would thus be of value in studying electrical phenomena arising in these regions.

Libet and Gerard,² working with the frog brain, have recently shown that the olfactory bulb generates a rhythm of its own which persists after separation from the rest of the brain and which may be detected in olfactory bulb fragments weighing as little as 0.1 mg. These authors³ have also put forward evidence for the existence of a potential gradient along an electrical axis, extending between the occipital pole at one end and the olfactory bulb at the other. It seemed to be worth while, therefore, to provide a technique permitting the study of electrical events occurring in the olfactory bulb in man.

The purpose of the present paper is to describe procedures for obtaining cerebral potentials from the surface of the eye and from the interior of the nasal cavity in the vicinity of the olfactory nerve.

The Electrodes.—Fig. 1a shows an ocular electrode which may be applied directly to the eyeball without the use of an anesthetic. It is composed of a gold shell *b* provided with a platinum stem *s*. A fine wire *w* leads off potentials from stem *s* to the amplifier. Shell portion *b* is shaped to reproduce the form and dimensions of the so-called "contact" glass lenses now in commercial use for correcting errors in refraction. It is of circular section and measures 21 mm. in diameter at the base and 8 mm. in height. Attempts were made to utilize glass contact lenses as electrodes by metalizing the interior and exterior surfaces, but because of difficulties in obtaining a firmly adherent metal film and the

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danger of breakage, the solid gold type of electrode was adopted. In applying contact lenses to the eye, it is usual to half fill the lens with 0.5 normal saline prior to insertion. This makes the lens adhere firmly to the eyeball. Comparative tests with and without 0.5 normal saline showed that satisfactory electrical contact of the electrode with the surface of the eye could be obtained without recourse to saline. Tests carried out on my eyes with and without an anesthetic (holocaine) also showed that there was little gain in comfort by using the anesthetic.

The intranasal electrode represented in Fig. 1b is composed of a length of ordinary solder wire *a* enclosed in a sheath of "spaghetti" insulation *Sp* up to a point about $\frac{1}{4}$ inch from one end. A small wad of absorbent cotton *c* is twisted over the active end of the electrode and held in place by several turns of ordinary sewing cotton. A fine wire *w*, soldered to the opposite end of the electrode, leads off to the amplifier.

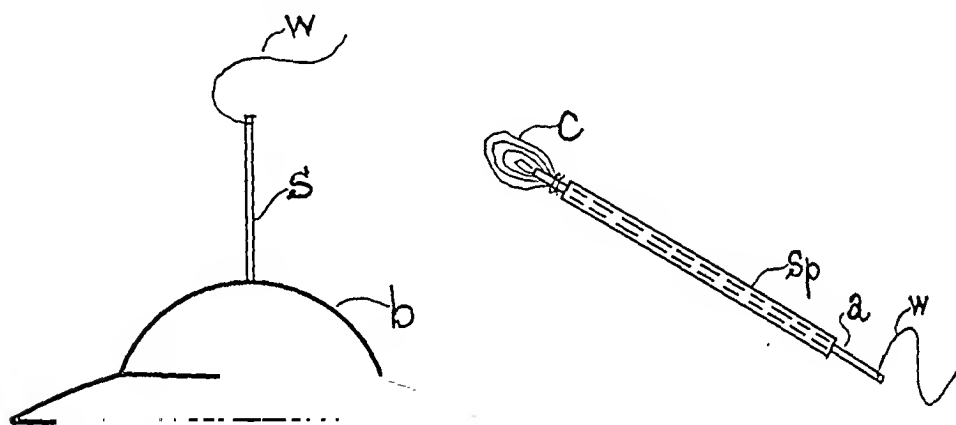


Fig. 1.—Electrodes for obtaining electroencephalograms: *a*, ocular electrode; *b*, intranasal electrode.

Experimental Procedure.—In preparing for electroencephalographic readings, with the subject in a sitting position, the usual lead pellet electrodes, their active sides covered with electrode paste, are first fixed in place by means of collodion in the occipital, frontal, and other regions to be explored with relation to the eye and nose. The subject is then placed in the decubitus dorsalis position, and the eye electrode is slipped into place between the eyelids. The subject is allowed to rest for a few minutes so as to become accustomed to the electrode and is instructed to seek a comfortable partially closed eyelid position which may be retained without blinking. This can be done by the average moderately relaxed type of subject without great difficulty. However, even in the best of subjects, occasional blink artifacts appear in the recordings, generally in groups, and are easily recognized by the appearance of large, rapid, potential changes.

Prior to placing the nasal electrode in position, the nasal cavity is swabbed first with 1:10,000 adrenalin solution, then with a 2 per cent solution of cocaine. The nasal electrode, moistened with normal saline, is then inserted as far as possible anteriorly, and is held in place by stuffing absorbent cotton into the free space between the electrode body and the nasal wall.

Records were made in a shielded, darkened room, using the two-channel, push-pull ink-writing recorder-amplifier described by Rahm.⁴ The time constant of the amplifiers was 0.5 second. Comparative tracings were made of eye occipital, nose occipital and fronto-occipital

potentials. These same potentials were also compared with those taken off from the occiput, forehead, eye and nose, using the lobe of the ear as a relatively inactive point of reference. The ear electrode was, however, not grounded. In order to insure against the possibility of coupling of the voltages led to the two amplifiers, leads feeding each channel were always taken from independent points. For example, if it was desired to compare the eye occipital with the ear occipital potentials, connections were made to the right occiput and the right eye for one channel, and with the left occiput and the ear for the other. Whenever possible, all leads were taken from the same hemisphere. The frontal and occipital electrodes were placed symmetrically at 3 cm. to the right and left of the midline. Only one ocular electrode

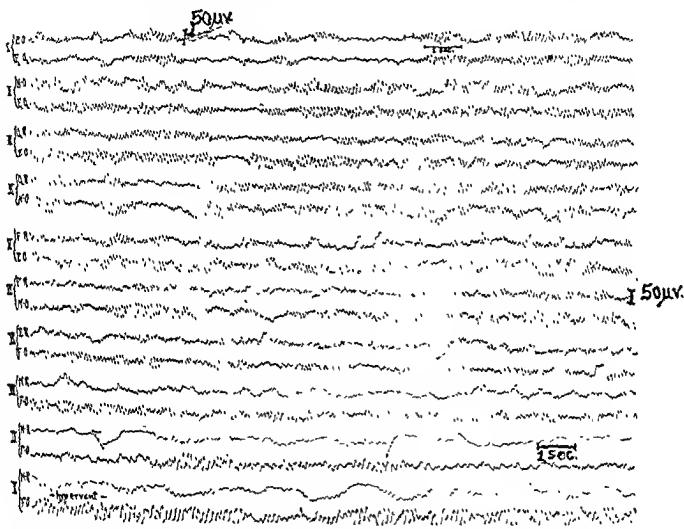


Fig. 2. Records I to X obtained with ocular and intranasal electrodes on a normal subject, and after hyperventilation on an epileptic patient. e, N, nose; R, ear; F, frontal; and O, occipital. All except those bearing the subscript L, which are from the left hemisphere.

- I. Eye occipital and fronto-occipital.
- II. Nose occipital and fronto-occipital.
- III. Occipital ear and eye occipital.
- IV. Occipital ear and nose occipital.
- V. Frontal ear and eye occipital.
- VI. Frontal ear and nose occipital.
- VII. Eye-ear and fronto-occipital.
- VIII. Nose-ear and fronto-occipital.
- IX and X. Nose-ear and fronto-occipital.

was available for use and was mounted on the right eye. Simultaneous left and right fronto-occipital records of the normal subject for whom results are shown in Fig. 2 showed negligible differences in phase, frequency, and amplitude. Studies of both nasal and ocular potentials were carried out on a total of six normal subjects.

RESULTS

Fig. 2, records I to VIII, inclusive, show typical tracings obtained on a normal female, D. D., aged 26 years, with the ocular and nasal electrodes described above using various electrode combinations. Records I and II show

eye occipital (E-O) and nose occipital (N-O) potentials, each taken simultaneously with a fronto-occipital (F_L-O_L) tracing serving for comparison. All these curves are of about the same amplitude and frequency, exhibiting an alpha rhythm of 10 per second. In order to obtain a better idea of the contribution of the ocular and nasal components to the total recorded potentials, tracings were made using occipital ear (O_L-R) and frontal ear ($F-R$) leads, each taken simultaneously with eye occipital and nose occipital records. As will be seen from tracings III to VI, inclusive, both the occipital ear and frontal ear potentials are of lesser amplitude than those obtained with eye occipital and nose occipital leads. Finally, in tracings VII and VIII, eye-ear (E-R) and nose-ear (N-R) potentials are each shown with simultaneous fronto-occipital records for comparison. Both the eye-ear and nose-ear tracings contain a certain amount of alpha modulated by a faster rhythm of about 25 per second of lesser amplitude. The proportion of fast component was found to vary considerably in different subjects. As will be seen by a comparison of tracings III to VI with VII and VIII, the amplitudes of the eye-ear and nose-ear potentials are considerably less than the corresponding frontal ear and occipital ear potentials. The eye-ear potentials are of the order of 15 to 20 microvolts, while the nose-ear potentials are about 10 to 15 microvolts. A typical blink artifact is shown in the eye-ear record of tracing VII.

Since, as has already been pointed out, the occipital pole and the olfactory bulb of the frog represent, according to Gerard and Libet,³ opposite ends of an electrical axis along which a potential gradient exists, an attempt was made to follow in parallel the effect of an electrical change on the occipital and nasal potentials. For this purpose, simultaneous tracings were made of the fronto-occipital and nasal-auricular potentials on T. R., a male epileptic (petit mal), aged 21 years, before and after mild hyperventilation. The results are shown in records IX and X. Prior to hyperventilation (IX), occasional bursts of alpha were picked up by the nasal electrode coincidentally with the appearance of similar bursts in the fronto-occipital leads. After hyperventilation (X) the fronto-occipital potentials show practically continuous alpha activity of high, nearly constant amplitude. The amplitude and percentage time alpha in the nasal tracing were found to increase to correspond to the alpha change in the fronto-occipital leads.

DISCUSSION

In the eye records action potentials arising in the extrinsic muscles of the eye or in the muscles of the eyelids would be due to such action currents as may be necessary to maintain these muscles at a relatively fixed tonus, since rotation of the eyeball or movements of the lids appear on the records as large, rapid potential changes. The frequencies of the action potentials from these muscles do not appear to have been reported in the physiologic literature. It is significant to note that the 25 per second rhythm appearing in the eye-ear record (Fig. 2, VII) is also found in the corresponding frontal and nasal records (V, VI, and VII). This would argue in favor of its being of cerebral rather than of muscular, origin. Jasper and Andrews⁵ have reported the occurrence of a beta rhythm of 25 per second of precentral frontal origin.

Grinker and Serota,⁶ using an electrode inserted through the anterior wall of the sphenoid sinus, describe a 4 to 6 per second rhythm of hypothalamic origin. A study of the various eye tracings which, as has been pointed out, might contain hypothalamic potentials taken off by the optic nerve, failed to show the presence of slow waves of this kind.

It would be hazardous to attempt to explain the origin of the alpha nasal potentials. With the electrode in contact with the intranasal ramifications of the olfactory nerve, the alpha potentials picked off may arise in the olfactory bulb, in the frontal lobes,⁵ or even be transmitted from the occipital cortical centers.⁷ The hyperventilation experiment merely shows a parallelism in the response under a nasal electrode and from fronto-occipital leads, but it does not permit conclusions as to interrelations of a controlling nature between the two.

SUMMARY

Techniques are described permitting electroencephalograms to be made using a metal "contact" lens type of ocular electrode and a nasal electrode of simple design. Potentials taken off between one ear lobe and the surface of the eye or the interior of the nose show the presence of alpha rhythms together with rhythms of higher frequency having the character of beta waves. The eye-ear and nose-ear alpha potentials are of lesser amplitude than those obtained using occipital ear and frontal ear leads. In an epileptic person marked changes in fronto-occipital potentials caused by hyperventilation were accompanied by parallel changes in nasal-auricular potentials.

REFERENCES

1. Williams, D., and Gibbs, F. A.: Electroencephalography in Clinical Neurology, Arch. Neurol. & Psychiat. 41: 519, 1939.
2. Libet, B., and Gerard, R. W.: Control of the Potential Rhythm of the Frog Brain, J. Neurophysiol. 2: 153, 1939.
3. Gerard, R. W., and Libet, B.: Control of Normal and "Convulsive" Brain Potentials, Am. J. Psychiat. 96: 1125, 1940.
4. Rahm, W. J.: Electroencephalography, Electronics, October, 1939.
5. Jasper, H. H., and Andrews, H. L.: Electro-encephalography: III. Normal Differentiation of Occipital and Precentral Regions in Man, Arch. Neurol. & Psychiat. 39: 96, 1938.
6. Grinker, R. R., and Serota, H.: Studies on Corticohypothalamic Relations in the Cat and Man, J. Neurophysiol. 6: 573, 1938.
7. Adrian, E. D., and Yamagiwa, D.: Origin of the Berger Rhythm, Brain 58: 323, 1935.

A METHOD FOR COUNTING THE LEUCOCYTES IN BLOOD CONTAINING GUM ACACIA*

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AFTER the injection of gum acacia into the blood stream it is not possible to obtain satisfactory leucocyte counts using the customary Tuerek's solution (1 per cent acetic acid) as the lytic agent of the red blood cells. Instead of a clear microscopic field containing only faint shadows of the red blood cells, one obtains, in the presence of gum acacia, an incomplete lysis of red blood cell structure, and an amorphous debris representing clumps of red blood cells in varying states of disintegration. This difficulty was first observed by Huffman¹ and was briefly investigated by Walker.² The latter stated that N/10 hydrochloric acid could be successfully substituted for the acetic acid.

Similar difficulties in obtaining satisfactory lysis of red blood cells were encountered in this laboratory when attempts were made to determine whether or not the leucocytes were effectively removed from the blood stream of animals subjected to complete plasmapheresis with gum acacia-red blood cell-Locke's solution. Although Walker described satisfactory results using N/10 hydrochloric acid on human blood containing gum acacia, further investigation on our part showed it altogether unsatisfactory with dogs' blood and only partially so in human blood. In the face of this difficulty, an attempt was made to find another hemolytic agent which would make possible the counting of the leucocytes under the conditions described.

METHOD

A series of hemolytic agents was prepared. The result obtained with each was compared with the optimal result obtained by the use of dilute acetic acid on normal blood. For each comparison two portions of the same sample of blood were centrifuged. The plasma of one portion was carefully removed and replaced with an equal volume of 6 per cent gum acacia dissolved in 0.9 per cent saline. Both portions of blood were then shaken vigorously for fifteen minutes. A standard diluting pipette (1:20) was used to prepare the dilutions of the blood samples for the counting chamber (standard American hemocytometer with Levy-Hausser counting chamber†). The criteria required of a satisfactory lytic agent were first, that it produce a clear microscopic field free of any cellular debris, and second, that it permit a series of cell counts, the average of which and the probable error of which, was the same as that of a normal control sample in which the usual Tuerek's solution had been used as the diluting and hemolyzing agent.

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†Manufactured by the Arthur H. Thomas Co., Philadelphia, Pa.

OBSERVATIONS

1. *Acetic acid*: Dilutions of acetic acid, ranging from 0.5 per cent to 5.0 per cent, were made and tested for their effect upon the lysis of red blood cells. Solutions of 0.75 to 1.0 per cent acid are the most satisfactory. Concentrations higher than this reduce the red blood cells to pointlike specks. When gum acacia is present in the blood, the laking of the red blood cells does not take place. Instead, large clumps of cells form as the diluting fluid is drawn into the pipette. Under the microscope, the white blood cells are difficult to find because of the irregular, coarse, amorphous collections of red blood cells and cell debris which occupy the field. Numerous individual red blood cells appear as pale brownish yellow spheres or as unorganized masses.

2. *Lactic acid*: Dilutions of lactic acid were also made. Using normal blood, this acid is equally as effective as acetic acid. Its optimal concentration is about 1.8 per cent. In blood containing gum acacia, however, the lysis of the red blood cells results in the same amorphous precipitate as that encountered with acetic acid.

3. *Hydrochloric acid*: No concentration of hydrochloric acid was found which gives as clear a differentiation of leucocytes in normal blood as does the 1 per cent acetic acid. Although the N/10 hydrochloric acid permits the count of leucocytes in fields of normal blood, the fields are frequently filled with indistinct dotlike forms which cannot be brought into clear focus. The gum-containing blood always possesses a more pronounced background of these small pinpoint bodies. The leucocytes in some samples of human blood may be easily counted, while other samples have too much debris and too great a density of dots in the background to permit more than a hazardous guess on the number of white blood cells present. With dog blood it is impossible to make reliable counts on any samples of blood containing the gum acacia.

4. *Phosphoric acid*: As an example of another inorganic acid, phosphoric acid was tried and, under the same conditions of normality as the hydrochloric acid, it was found to be a somewhat better lytic agent for the normal red blood cells. However, it too is an unsatisfactory hemolytic agent in bloods that contain gum acacia.

5. *Saponin*: Since neither organic nor mineral acids promised to produce desirable results, a different type of lytic agent was investigated. It was found that the lysis of red blood cells, either in normal or in gum-containing bloods, was complete when the glycoside, saponin, was used. Saponin alone, however, did not produce a satisfactory microscopic field even though a range of solutions from 0.05 per cent to 5.0 per cent was investigated. The white blood cells appeared small and misshapen. Addition of any of the acids previously tested immediately produced the usual bad effect. Normal saline was finally added on the assumption that it would protect the white blood cells against undue distortion resulting from the osmotic effect of a hypotonic solution. A solution of 0.1 per cent saponin and 0.9 per cent saline was found to be quite satisfactory. The white blood cells were vitally stained by adding methyl violet in a concentration of 0.1 per cent to the saponin-saline solution. When two fields of normal blood, one diluted with acetic acid, and the other with a saponin-saline solution,

were compared side by side, the white blood cell counts of each were found to agree with a precision within the range of probable error of the measurement. When the leucocyte counts were made on blood containing gum acacia, they were found not only to be made easily, but also to be identical with those made on samples of the same blood containing no acacia. It was found that the rate of lysis by saponin is proportional to the concentration of the lytic agent, and to the number of cells. The solution of 0.1 per cent saponin in 0.9 per cent saline requires about five minutes for complete lysis of all red blood cells. This action may be watched under the microscope and the time of complete lysis may be readily determined.

In addition to the three types of lytic agents discussed, the simple osmotic lysis of "gum acacia" cells in hypotonic solutions of sodium chloride was also studied. The degree of hypotonicity at which red blood cells rupture was found to be unaffected by the presence of gum acacia, regardless of whether the gum had been added to the cells *in vitro* or *in vivo*.

DISCUSSION

No satisfactory explanation for this failure of the common lytic agents to cause the lysis of red blood cells, suspended in gum acacia, can be deduced from the data collected.

Simple lysis of red blood cells by hypotonic solution of inorganic salts is the result of osmotic equilibration.⁵ Variation in the rate of lysis by different inorganic salts is related only to the relative speeds of diffusion of the ions concerned across the plasma membrane. As mentioned in the previous paragraph, this action is not hindered by the presence of gum acacia.

Inorganic acids apparently cause lysis by some action other than an osmotic one. Bodansky³ lists phosphoric, sulfuric, hydrochloric, and nitric acids in the order of decreasing effectiveness as agents in the lysis of red blood cells. This investigator states that the pH is not the only factor concerned in the lytic action and that the effect is primarily not an osmotic one. More likely it is a reaction upon one of the units of structure in the cell membrane. This unit may very probably be the protein molecule, the orientated structure of which is destroyed by the acid. Gum acacia definitely interferes with this action.

The organic acids cause lysis of the red blood cell by a modified osmotic action.⁴ Increasing the osmotic pressure of the diluting fluid decreases the effectiveness of the lytic agent, but a close relationship is found between lipid solubility and the hemolytic activity of the organic acid. The higher the molecular weight and the more lipid soluble the acid, the more effective is the acid in equivalent concentrations. Gum acacia interferes markedly with this type of lysis.

Saponin represents a fourth type of lytic agent. "The fundamental action of a simple lysin such as saponin is imagined to be one in which the lysin reacts with some component of the red cell membrane, and which continues after lysis is complete until the cell component concerned is completely transformed as a result of a union with the lysin."⁵ Saponin causes an initial hemolysis followed by a complete stromatolysis. The simple lytic effect of a hypotonic solution is nonadditive to the more complex saponin lysis, implying an independence of the

two processes.⁶ Gum acacia does not affect this action. If acids are added to the saponin, the same precipitation is encountered which is observed when the acids are used alone. Saponin, acting on the lecithin portion of the stroma, may completely disorganize the cell and stroma. Inorganic acids acting on the cell membrane may act only on the protein structure in the membrane, causing their denaturation. The organic, fat-soluble acids, on the other hand, may not permeate so freely in the presence of gum acacia and thus not cause the expected hemolysis. The gum may act to change the surface tension of the stroma in the process of stromatolysis and thus effect the clumping of the cellular fragments.

Englebreth and Smith⁷ have also suggested the use of saponin as a lytic agent of red blood cells in the preparation of blood samples for leucocyte counts, but for a different reason. They call attention to the fact that white blood cells in citrated or heparinized blood may undergo a 20 per cent autolysis within several hours after drawing of the blood sample. This autolysis of the cells does not occur in the presence of 2 per cent sodium fluoride, indicating that the autolysis is caused by some enzyme, the activity of which is inhibited by the fluoride.

Fluoride of this strength, however, is not hypotonic enough to lysis the red blood cells. To overcome this difficulty, the authors employed a solution of 0.1 per cent saponin as a hemolytic agent. A solution of 0.1 per cent saponin, 1 per cent sodium fluoride, and 0.1 per cent methyl violet preserved the white blood cells so well that twenty-four hours after the mixing of the blood sample with the solution leucocyte counts could be made which were identical with those made immediately after the mixing.

SUMMARY

Leucocyte counts on bloods containing gum acacia are very difficult to make when the usual Tuercik's solution is used as the diluting and hemolytic agent. A solution of 0.1 per cent saponin, 0.9 per cent saline, and 0.1 per cent methyl violet is recommended instead. When this solution is used, complete hemolysis and stromatolysis of the red blood cells are obtained. The white blood cells are easily counted in both normal and acacia-containing bloods. The known factors concerning this lysis of red blood cells by various agents are discussed briefly.

REFERENCES

1. Huffman, L. D.: Solution of Acacia and Sodium Chloride in Hemorrhage and Shock, *J. A. M. A.* 93: 1693, 1929.
2. Walker, M. A.: Tenth-normal Hydrochloric Acid as a Diluent for Counting Leucocytes—After Infusion of Solution of Acacia, *Am. J. Clin. Path.* 2: 347, 1932.
3. Bodansky, M.: Lipoid Solubility, Permeability and Hemolytic Action of Saturated Fatty Acids, *J. Biol. Chem.* 79: 229, 1928.
4. Bodansky, M.: The Hemolytic Action of Inorganic Acids, *J. Biol. Chem.* 79: 241, 1928.
5. Ponder, E.: The Mammalian Red Cell and the Properties of Hemolytic Systems, Berlin, 1934, Brontraeger, pp. 117, 272.
6. Ponder, E.: Effect of Simple Hemolysins in Hypotonic Systems, *Protoplasma* 27: 523, 1937.
7. Englebreth, H. J., and Smith, C. C. W.: A Source of Error in the White Blood Counts, *Acta med. Scandinav.* 95: 129, 1928.

AN AUTOMATIC PIPETTE AND A NEW INEXPENSIVE PIPETTING MACHINE*

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THE bane of the serologist is the large amount of tedious pipetting involved in complement fixation tests, such as Wassermann reactions. Pipettes must be filled often, the meniscus carefully followed, and any attempt at speed often results in inaccuracies. Pipetting is a skilled procedure and unless one has had much experience, fatigue and cramps of the fingers slow up the process and tend to produce errors.

Attempts to reduce the inherent disadvantages of pipetting have resulted in the development of more or less automatic pipettes. For the most part these consist of syringes adjustable to deliver a given quantity of fluid. One type is operated manually and is filled by the recoil of a spring attached to the plunger. The amount of fluid delivered by the syringe is adjusted by a stopping device which limits the amount of material drawn into the apparatus. While this type of pipette increases the speed of pipetting over the usual glass pipette, there is still a loss of time in conveying it back and forth from the vessel containing the stock of reagent to the test tubes. The speed of pipetting depends on the development of coordination between this back and forth movement and the alternate compression and release of the plunger.

Another type of mechanical pipetting device is the so-called pipetting machine which is an adaptation of the original Woodyatt machine devised for the continuous intravenous injection of fluids at constant rates. It is likewise a syringe whose plunger is operated by an eccentric turned by an electric motor which also synchronizes a two-way valve. The amount of fluid delivered is adjusted by the length of stroke of the plunger, and the speed of operation is determined by the adjustable speed of the motor. The greatest objection to this device is its cost. Also, if more than one fluid is to be pipetted and unless more than one machine is available, it must be cleaned with each change of fluid and the mechanism readjusted if the volume to be delivered is also changed, both of which are time-consuming procedures.

Beeton, Dickinson & Co. manufacture an automatic syringe of the former type which is described in their literature as the "Improved Cornwall Syringe Number 1250." On request, this company interposed between the barrel of the syringe and the trocar, used as a pipette tip, a small two-way valve. This valve has a female "Luer-Lok" that connects with the syringe and a male "Luer-Lok" that takes the trocar. A side arm of the valve is fitted with a rub-

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ber tubing that leads to the reservoir of the fluid to be pipetted. At the distal end of this tube is a perforated metal ball which keeps the tube submerged in the fluid. The apparatus is essentially a 2 c.c. Luer syringe mounted in a metal shell. The top of the plunger is attached by means of a yoke to a screw which

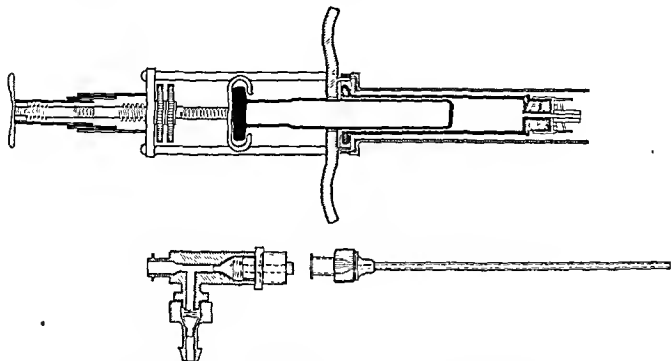


Fig. 1.—Diagram showing construction of automatic pipette.

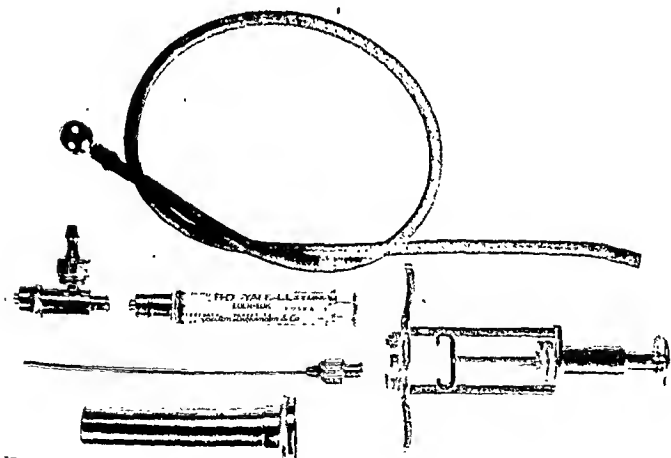


Fig. 2—Separate parts of automatic pipette.

carries the plunger up and down. Its upward motion is given by a spring around the screw and is stopped by two lock nuts. The downward motion is obtained manually and is opposed to the force of the spring. The upward or filling stroke closes a valve leading to the trocar and opens a valve leading to the rubber tube and reservoir. The downward or emptying motion has the reverse

effect. Fig. 1 is a diagram showing the construction of the syringe. Fig. 2 is a photograph of the separate parts and, Fig. 3, of the whole assembled for use.

In adjusting the amount of fluid to be delivered the syringe is emptied 10 or 20 times into a small graduate. If this delivered amount is too small or too large, the plunger upstroke is adjusted by the lock nuts until it is correct. Once adjusted the amount delivered is remarkably constant.

By actual test it is found that from one-fifth to one-tenth of the pipetting time used in complement fixation tests with the ordinary pipette is used with this apparatus. No previous experience in pipetting is necessary for its use. A little practice with it enormously increases the speed. The operator simply aims the tip at the mouth of the tube, compresses the plunger, releases it, and it is ready for the next tube. Another advantage observed is that the fluid is forced into the test tubes rather than allowed to flow in as with an ordinary pipette. This completely mixes the fluids in the tubes and makes shaking unnecessary.

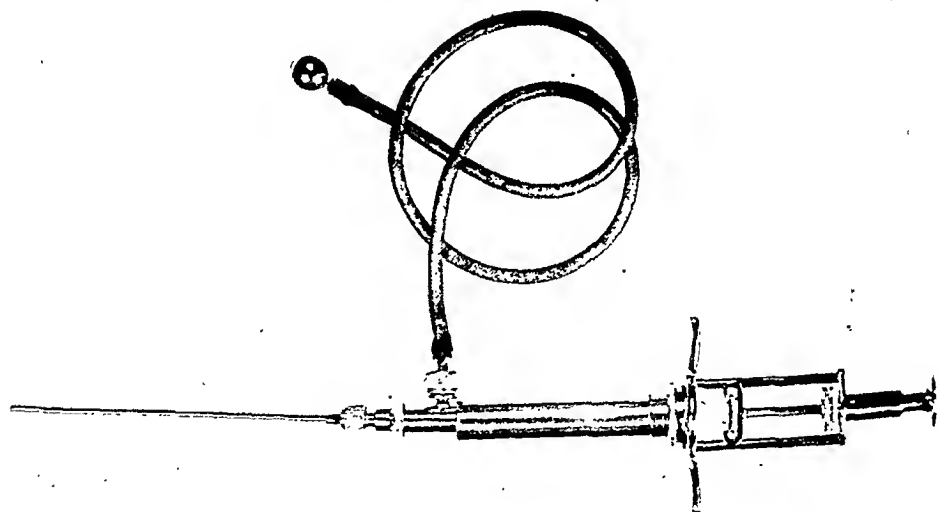


Fig. 3.—Automatic pipette assembled for use.

In the Kolmer complement fixation test for syphilis, five reagents, exclusive of the patients' sera, are used: saline, complement, antigen, hemolytic amboceptor, and red blood cells. The saline is used in two quantities, 1.2 c.c. and 0.5 c.c.; antigen is used in 0.5 c.c. amounts; complement in 1 c.c. amounts; hemolysin and red blood cells each in 0.5 c.c. amounts. For the greatest speed one may use one syringe for each of the reagents, two for the two quantities of saline, necessitating altogether six syringes. The price of the apparatus is reasonable enough to afford this. An alternative is to use three syringes, one for each of the reagents used in the same quantity. One, adjusted to deliver 0.5 c.c., may be used for saline, antigen, hemolysin, and red blood cells; an-

other, adjusted to deliver 1.0 c.c., for complement; and the third, adjusted to deliver 1.2 c.c., for saline. This necessitates cleaning the apparatus when solutions are changed.

Cheap Motor for the Automatic Syringe.—An apparatus for actuating the above syringe was devised using a compressed air type windshield wiper motor. The shaft of the motor, which is mounted on a baseboard, is fitted with a slotted arm to which is attached a connecting rod to a shaft housed in a bronze bearing. The end of the latter impinges against the plunger end of the above automatic syringe which is mounted on a block (Fig. 4). The stroke of the shaft is adjustable at the slot of the arm on the motor. The energy of the motor is used only for

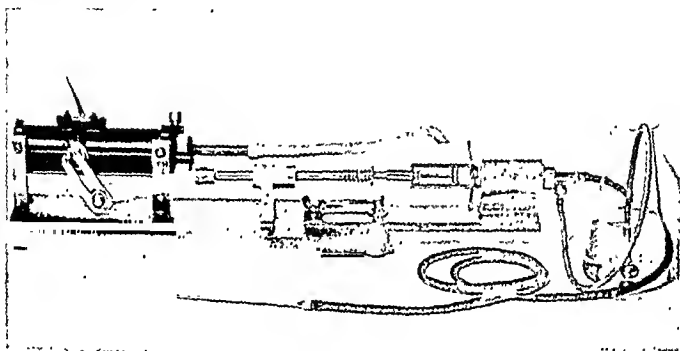


Fig. 4.—Pipetting machine using windshield wiper as the motor.

emptying the syringe, its own spring bringing about the refill. The shaft that impinges against the syringe is broken at its end, and the parts are united with a coiled spring which takes up a slight overstroke and thus protects the syringe. A thick-walled rubber tubing of small caliber is attached to the two-way valve, and to this is fastened the trocar to serve as a delivery tip. A wide range of speeds is obtainable by adjusting the pressure of air to the motor.* This apparatus will, in every respect, give the same efficient pipette service as the much more expensive electrically operated pipetting machine. As the Woodyatt machine, designed for intravenous injections, was adapted for pipetting, so this apparatus, made for pipetting, can equally well be used for injecting.

*This apparatus was constructed in the machine shop of the Otho S. A. Sprague Memorial Institute.

AN IMPROVED TECHNIQUE FOR PNEUMOCOCCUS TYPING BY THE "QUELLUNG" REACTION*

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THE "Quellung" reaction is the method of choice for typing pneumococci in sputum because of its time-saving element.

The following modification of the original method has been used by us for the last three years. We believe its advantages make it worth reporting.

Technique.—From the mucoid rather than from the purulent portion of the sputum four very thin smears are made in the form of a ribbon 10 to 15 mm. wide along the long axis of microscopic slides. The smears are allowed to dry at room temperature. Usually the first smear is dry when the fourth smear is completed. Two of the smears are marked with the group designations A, B, C, D, E, and F. One loopful of group A antipneumococcal serum is spread on the smear in a round area about 10 mm. in diameter. If methylene blue has been added to the antiserum previously, two loopfuls are used, otherwise another loopful of methylene blue is spread over the antiserum. A round cover glass, 18 mm. in diameter, is used to cover this area. No vaseline or jelly is needed. Similar smears are made with antisera of group B and C on the same slide; groups D, E, and F, are put likewise on a second slide and properly marked. These smears can be examined immediately under the 4 mm. objective.

COMMENT

The dry smear presents the following advantages: it affords better concentration of the antiserum, the microscopic field looks clearer, and additional smears can be made and saved for reference. (Our three-year-old smears still give excellent reaction.) In cases where sputum cannot be obtained, throat swabbings can be smeared on slides at the bedside, dried, and sent to the laboratory. Physicians in rural communities, where laboratory facilities are not available, can mail these dried smears to any laboratory. In our experience mailing of sputum has been very unsatisfactory. Dried smears can also be utilized in schools and colleges for teaching purposes. Hundreds of slides can be made from typed sputum and utilized for classwork whenever needed.

The advantages of the 4 mm. objective over the oil-immersion objective are twofold: (1) More area is covered in a shorter time, facilitating quick diagnosis. (2) The microscopic field can be studied with greater ease without disturbing the picture because the objective does not touch the smear. With oil-immersion objective the focusing throws the organisms off focus, or accompanying changes of pressure create currents which carry the organisms out of the field.

The Michigan State Board of Health Laboratories have adopted the above-described method. It is reported that the New York State Laboratories have also adopted it.

*From the Pathological Laboratory, Evangelical Deaconess Hospital, Detroit.
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CHEMICAL

THE CONVERSION OF CREATININE INTO CREATINE IN NORMAL URINE*

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IN CONNECTION with studies conducted in this laboratory that required twenty-four-hour collections of urine, some of the analyses were not made until two weeks after the samples had been collected. The finding of quantities of creatine greater than that recorded in the literature (Hobson, 1929) led us to suspect that conversion of creatinine to creatine had occurred, even though the samples of urine had been preserved with thymol and kept at room

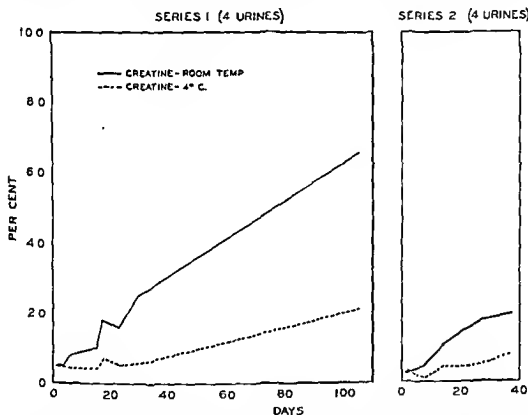


Fig 1—Curves showing the average increase in the percentage of creatine (as creatinine) of total creatinine with time when urine is stored at room temperature and at 4° C. Series 1 represents urines collected in May, and series 2 represents urines collected in August.

temperatures. Such proved to be the case. On one patient three urines that had been analyzed fifteen days after collections were again analyzed after forty-one days at room temperatures. The average creatine percentage (expressed as creatinine) in total creatinine substances had increased from 7 to 18. The average values in the urines of two other men showed changes from 4 to 13 and from 12 to 25 per cent during twenty-six days following the first analysis.

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We proceeded to study this problem in more detail. Since the storage temperature might be of importance, freshly voided urine was preserved with thymol and placed in the refrigerator at about 4°C . The volumes of the twenty-four-hour outputs were recorded, and two aliquots, also preserved with thymol, were taken. One was returned to the refrigerator, the other was left at room tem-

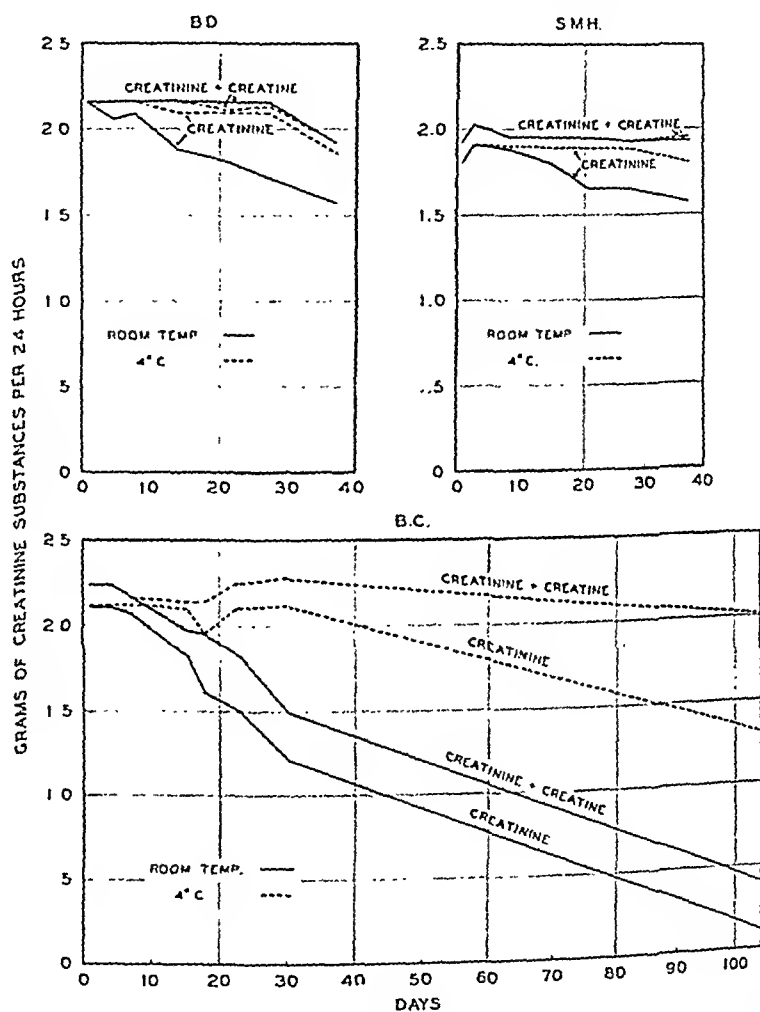


Fig. 2.—Curves illustrating various types and rates of conversion in aliquots of urine stored at room temperature and at 4°C .

peratures. Determinations of creatinine and of creatine plus creatinine (Folin, 1934), using the Evelyn photocolormeter, were made immediately and repeated at intervals thereafter. Creatine, expressed in terms of creatinine, was obtained by difference. Two series of experiments on a total of 4 persons were conducted, one starting in May and the other in August.

RESULTS

The data are presented graphically (Figs. 1 and 2). Total nitrogen determinations were made by the Kjeldahl method at the beginning and end of the experiments; no changes were noted. Aliquots kept at 4°C . maintained nearly

constant creatinine and creatine contents. In only 2 of 8 cases were creatine variations greater than 100 mg. found after standing in the cold for thirty days. After storage in the cold for 104 days, greater conversion occurred, amounting to from one-fifth to one-third of the total creatinine in three of the four samples of series I. In series II analyses on the thirty-seventh day showed less conversion of creatinine into creatine than was observed in series I after thirty days.

The aliquots kept at room temperature usually, but not always, showed a constant total creatinine. The urines of S. M. H. and B. C. (series I) gradually decreased in total creatinine with a concomitant rise in creatine. This was particularly apparent after a period of 104 days, when in B.C. the total creatinine was one-fifth its initial value (2.24 to 0.43 gm.), while the creatine value increased more than 2.5 times (0.12 to 0.33 Gm.) (Fig. 2). Analyses of other urines gave no greater variations in total creatinine than are within the accuracy of the method. Creatine values increase not only in per cent of total creatinine (Fig. 1) but also in actual amounts if kept at room temperature for more than nine days. This conversion occurs even when initial determinations gave zero values for creatine (Fig. 2).

Heintz (1847) showed that when he made preparations of creatinine from urine, creatine contaminated all his preparations. Liebig (1847), however, was only able to get creatinine from urine and showed that it was the use of ammonia and its compounds by Heintz that decomposed his creatinine zinc chloride to creatine. Dessaignes (1857) noted a slow conversion of creatinine to creatine, hastened by heat, to occur in aqueous solutions. The reversal of this reaction has also been recorded (Neubauer, 1866; Hahn and Barkan, 1920). Myers and Fine (1918) have recorded a gradual production of creatine in a pure aqueous solution of creatinine. Evidently heat, and perhaps ammonia, favor the conversion of creatinine to creatine; on long standing this may become appreciable.

SUMMARY

There is at first a slow and later a rapid conversion of creatinine if urine preserved with thymol stands at room temperature. At 4° C. conversion was observed on the thirty-seventh day and was pronounced by the one hundred and fourth day. While urine can be stored for a few days in the cold without appreciable change in either creatine or creatinine, it is a better practice to make these determinations within twenty-four hours after collecting the sample. In any case, preservation with thymol and storage in the cold are recommended.

REFERENCES

- Dessaignes, W.: *J. Pharm. Chem.* 32: 37, 1857.
 Folin, O.: *Laboratory Manual of Biological Chemistry*, ed. 5, New York, 1934, D. Appleton-Century Co.
 Hahn, A., and Barkan, G.: *Ztschr. f. Biol.* 72: 25, 1920.
 Heintz, O.: *Poggendorff's Ann. Physiol. Chem.* 62: 602, 1847.
 Hobson, W.: *Biochem. J.* 33: 1425, 1939.
 Liebig, J.: *J. prakt. Chem.* 40: 288, 1847.
 Myers, V. C., and Fine, M. S.: *J. Biol. Chem.* 17: 65, 1914.
 Neubauer, C.: *Ann. Chem. Pharm.* 137: 288, 1866.

URINARY PORPHYRIN BEFORE AND AFTER HYDROLYSIS*

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VERY little information is available about the form in which porphyrins are excreted in the urine. As a rule, no porphyrin can be extracted from urine by organic solvents prior to acidification with acetic acid. This basic step in urinary porphyrin analysis was first described in 1896 by Sallet,¹ who also noted the occurrence in urine of precursors of porphyrin—"chromogens"—which, although observed by many investigators,²⁻¹⁰ seem not to have been systematically taken into consideration when studying urinary porphyrin excretion in health and disease.

The extraction of zinc and copper complexes of porphyrins from urine acidified with acetic acid has been recorded.^{3, 11} The fact that no porphyrin can be extracted by ether from fresh urine of about pH 6 can be interpreted as evidence of conjugation. Assuming that the porphyrinogens are compounds of porphyrins with other inorganic or organic substances and differ in their stability to acetic acid, one may reasonably expect that hydrochloric acid would be more effective in bringing about a complete cleavage. Preliminary experiments have indicated that a widely varying rise occurs in the porphyrin level of urines on treatment with strong hydrochloric acid (see Tables I and II). The ratios of the porphyrin values so obtained varied widely for different persons, but were constant and in general reproducible for the same individual.

TABLE I
INCREASE IN COPROPORPHYRIN YIELDS AFTER HYDROLYSIS
(In micrograms per twenty-four-hour specimen of urine)

PATIENT	DISEASE	BEFORE HYDROLYSIS	AFTER HYDROLYSIS
1	Arsenical dermatitis	146	225
2	Lupus erythematosus	73	81
3	Hepatitis	224	310
4	Urticaria due to light	82	97
5	Undiagnosed	40	114
6	Undiagnosed	None	22
7*	Disseminated lupus erythematosus (photosensitive)	177	300
7*	Disseminated lupus erythematosus (photosensitive)	102	149
8	Addison's disease	116	322
9	Dermatitis of unknown origin	96	140
10	Dermatitis of unknown origin	56	100
11*	Nicotinic acid deficiency	188	228
11*	Nicotinic acid deficiency	176	220
11*	Nicotinic acid deficiency	184	228
12	Undiagnosed disease of skin	46	55

*These specimens were submitted on different dates.

*From the Departments of Biochemistry and Dermatology of the College of Physicians and Surgeons, Columbia University.

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TABLE II

INCREASES OF TWENTY-FOUR-HOUR URINARY COPROPORPHYRIN AFTER HYDROLYSIS IN A CASE OF ADDISON'S DISEASE

DATE	24-HOUR VOLUME (c.c.)	BEFORE HYDROLYSIS (μ g)	AFTER HYDROLYSIS (μ g)
8/2/40	2,990	45	119
8/3/40*	2,004	None	36
8/4/40*	2,155	15	48
8/5/40	1,580	55	100
8/6/40	1,430	38	85
8/7/40	1,435	36	78
8/8/40	1,600	42	83
8/9/40	2,690	31	97

*Attack of indigestion.

EXPERIMENTAL

After the hydrolysis of the hypothetical chromogens with hydrochloric acid, it was necessary to neutralize the solution with alkali and then to acidify it with glacial acetic acid prior to the extraction of coproporphyrin with ether. The treatment with hydrochloric acid considerably reduces the formation of disturbing emulsions during the ether extraction. However, it brings about a formation of urinary pigments other than porphyrin,^{12 13} and these must be separated before the spectroscopic identification and estimation of porphyrin can be carried out. Indirubin and indigo blue, which are simultaneously extracted with coproporphyrin by ether from the suitably prepared acetic acid solution, can be removed by means of 5 per cent hydrochloric acid, which extracts only the coproporphyrin.

Two types of experiment were performed systematically: Two 300 c.c. portions of each urine specimen are used for the determination of the urinary porphyrins. One is acidified with 30 c.c. of glacial acetic acid and the other with 30 c.c. of concentrated hydrochloric acid, and both are allowed to stand for at least two hours. The hydrochloric acid treated specimen is then made alkaline with potassium hydroxide and acidified with glacial acetic acid. Tap water is used to cool the solution during these operations. Both specimens are now treated in the same way, according to the accepted methods (Fischer¹⁴ and Rimington¹⁵). Each solution is shaken with three successive portions of ether; the combined ethereal solutions are washed first with water and then repeatedly with 5 to 10 c.c. quantities of 5 per cent hydrochloric acid to recover the porphyrin (coproporphyrin). This hydrochloric acid solution is neutralized and acidified with glacial acetic acid, and coproporphyrin is again taken up in ether. This ether extract is made up to a known volume in a 25 c.c. graduated cylinder; the porphyrin rarely requires further purification.

The spectroscopic examination was made with a Bausch & Lomb wave length spectrometer, essentially as described by Kapp and Coburn,¹⁶ hematoporphyrin, which spectroscopically resembles coproporphyrin very closely, being employed as a standard.¹⁷ When the spectra (from two light sources) of hematoporphyrin and coproporphyrin in ether, as well as in hydrochloric acid solution of about the same concentration, were superimposed, using the comparison prism of the Bausch & Lomb Laboratory wave length spectrometer, no difference could be seen.¹⁸

A series of standard hematoporphyrin solutions in ether were prepared, and the limits of their absorption bands were recorded. Two spectroscopic charac-

teristics of coproporphyrin were found to be particularly useful for the quantitative estimation of urinary coproporphyrin: a relatively faint band at about 5980 Å indicating the presence in 16 c.c. (200 mm. cell) of at least 14 micrograms, and the coproporphyrin band in the red at 6230 Å indicating the presence of at least 3 micrograms. The point at which an absorption band is just visible can be reached by suitable dilutions, or by using cells of different lengths.

Uroporphyrin was isolated from the acetic acid aqueous phase remaining after ether extraction of coproporphyrin. This residual solution was slowly passed through an adsorption column of Merek's aluminum oxide and eluted with 12 per cent ammonia.¹⁹ Uroporphyrin is not found in normal urine and no uroporphyrin standards are, as a rule, available. In the case of a patient with light sensitivity the ammoniacal uroporphyrin solutions obtained by elution from hydrolyzed and unhydrolyzed identical aliquot portions of urine when compared colorimetrically showed a threefold increase of uroporphyrin in the hydrolyzed portion.

SUMMARY

Increased yields of urinary porphyrin can be obtained by hydrolysis with hydrochloric acid.

By comparison of the porphyrin yields from unhydrolyzed and hydrolyzed samples of the same urine, it is possible to determine the ratio of total porphyrin to porphyrin present in form of porphyrinogen or chromogen.

REFERENCES

1. Süllet: De l'urospectrine ou urobilinoporphyrine normale. . . , *Rev. de méd., Paris* 16: 542, 1896.
2. Günther, Hans: Die Bedeutung der Hämato porphyrine in Physiologie und Pathologie, *Ergebn. d. allg. Path. u. path. Anat.* 20: 618, 1922.
3. Schumm, O.: Die spektrochemische Analyse natürlicher organischer Farbstoffe, Jena, 1927, Gustav Fischer, pp. 161, 192.
4. Sachs, P.: Ein Fall von akuter Porphyrie mit hochgradiger Muskelatrophie, *Klin. Wchnschr.* 10: 1123, 1931.
5. Rimington, C., Chaudler, F. G., and Harrison, G. A.: Clinical Porphyrinuria With Report of a Case of the Acute Idiopathic Type, *Brit. M. J.* p. 1173, 1939.
6. Watson, Cecil James: The Porphyrins and Their Relation to Disease: Porphyrin, Oxford Medicine (Christian) 4: 8, 1938.
7. Waldenström, J.: Studien über Porphyrie, *Acta med. Scandinav. Suppl.* 82: 53, 1937.
8. Mertens, E. J.: Ueber das Uroporphyrin bei akuter Hämato porphyrin, *Ztschr. f. physiol. Chem.* 238: 1, 11, 1936.
9. Vannotti, A.: Porphyrine und Porphyrinkrankheiten, Berlin, 1937, Julius Springer, p. 275.
10. Dobriner, K., and Rhoads, C. P.: The Porphyrins in Health and Disease, *Physiol. Rev.* 20: 437, 1940.
11. Kapp, E. M.: Spectrographic Observations on Urinary Porphyrins: The Nature of a Combined Form of Coproporphyrin Found in Urine, *Brit. J. Exper. Path.* 20: 33, 1939.
12. Garrod, A. E., and Hopkins, F. G.: On Urobilin, Part One. The Unity of Urobilin, *J. Physiol.* 20: 112, 1896.
13. Zeyneck, R. von: Harn und Blutfarbstoffe und deren Chromogene, sowie Melanine, in Carl Neuberg's *Der Harn*, Berlin, 1911, Julius Springer, p. 877.
14. Fischer, Hans: Neuere Methoden der Isolierung und des Nachweises von Porphyrinen, *Abderhalden's Handb. d. biol. Arbeitsmeth.* Abt. 1, Teil 11, 1936.
15. Rimington, C., and Hemmings, A. W.: Porphyrinuria Following Sulphanilamide: Sulphanilamide Dermatitis, *Lancet* 1: 770, 1938.
16. Kapp, E. M., and Coburn, A. F.: Studies on the Excretion of Urinary Porphyrin in Rheumatic Fever, *Brit. J. Exper. Path.* 17: 255, 1936.
17. Lageder, K.: Klinische Porphyrinuntersuchungen mit einer quantitativen spektroskopischen Methode, *Arch. f. Verdauungskr.* 56: 237, 1934.
18. See Watson reference 6, page 3.
19. Waldenström, J.: Untersuchungen über Harnfarbstoffe, hauptsächlich Porphyrine mittels der chromatographischen Analyse, *Deutsches Arch. f. klin. Med.* 178: 38, 1935.

PREPARATION OF CHOLESTEROL EMULSION FOR EXPERIMENTAL INTRAVENOUS ADMINISTRATION*

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THE oral and intravenous methods of administration of cholesterol known to us will not produce a significant elevation of the blood cholesterol. After experimentation with variations in technique of preparation of emulsions, we were able to prepare emulsions in concentration as great as 2, 3, or 4 per cent by dispersion of the emulsion in steam. No claims for priority are made, since the method is perhaps used extensively commercially. Homogenization, a principle commonly used in the dairy industry, is a very efficient method of producing emulsions, but the homogenizing machines usually available are so large that several gallons are necessary for their function, thereby making the process for our purpose prohibitive financially. Moreover, preheating of the ether-oleate-cholesterol solution, as utilized in our method, seems important in the emulsification, and might be difficult to attain in a homogenizer.

METHOD

1. Dissolve 3 Gm. of cholesterol in about 50 c.c. of ethyl ether.
2. Dissolve 1 Gm. of sodium oleate in sufficient water to make 100 c.c. In our experience tap water is *much superior* to distilled water, as far as the emulsification process is concerned.
3. To solution No. 2, contained in a 500 c.c. Erlenmeyer flask, slowly add solution No. 1, stirring or swirling constantly. This ether-oleate-cholesterol solution is now ready for dispersion, which is brought about by allowing a small stream of the ether-oleate-cholesterol solution to come in contact with a jet of steam at the end of a T-tube, as illustrated in Fig. 1.

The apparatus herein described can be set up with equipment found in practically any laboratory. A pressure cooker or small autoclave (Fig. 1) is heated until a pressure of 15 or 20 pounds is obtained. The valve is opened and the steam is allowed to pass through the T-tube surrounding the tube which conducts the cholesterol solution. After the glassware is hot, the ether-oleate cholesterol solution is allowed to run out; it is heated by the steam surrounding the glass tube conducting it, and flows out of the 1 mm. opening as a fine spray, coming in immediate contact with the steam emerging from the tip of the outside tube. It is important that the size of the steam spray be adjusted by moving the inside tube very slightly in or out, until a mixture is obtained producing a fine emulsion. The emulsion can be tested for size of the globules

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in a few moments by observation under the microscope. When first using the apparatus, it will be necessary to experiment considerably with the size of the steam jet before a satisfactory emulsion with globules less than 4 or 5 microns in diameter will be obtained. Passage of the emulsion through the apparatus a second time will improve the emulsion. Preliminary to the second dispersion the inside tube is pushed forward, narrowing the steam outlet. It is not wise to pass the solution through more than twice, since the volume will be increased by condensation of steam, thereby cutting down on the concentration. After a little experience with the apparatus, it will be found that the concentration of cholesterol in solution No. 1 can be increased beyond 6 per cent.

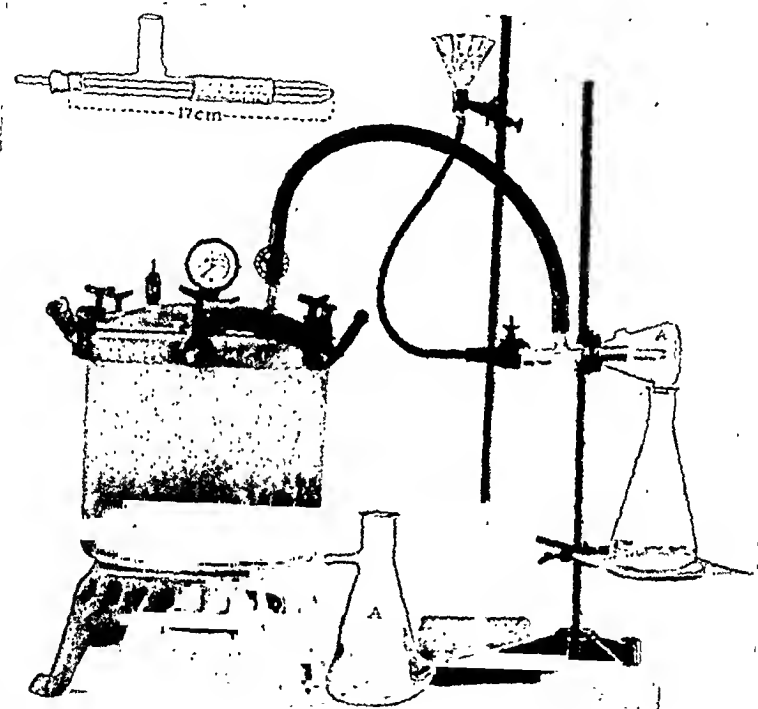


FIG. 1.—Apparatus used in emulsifying cholesterol. By means of a pressure cooker or autoclave, steam is forced through a T-tube coming in contact with the ether-oleate-cholesterol solution as it emerges through the tip of the small tube within the T-tube. Insert represents a longitudinal section of the T-tube and illustrates how steam entering at the arm of the T-tube heats the ether-oleate-cholesterol solution and emulsifies it at its exit. The outside diameter of the inside tube conducting the ether-oleate-cholesterol solution is 5 mm. The end of the inner tube has previously been drawn to a point in a flame; the diameter of the opening at the end of this tube, where the solution emerges, is about 1 mm. This bevel at the end of the inner tube allows it to be pushed toward the end of the T-tube until a very small rim of space remains through which the steam emerges to come in immediate contact with the ether-oleate-cholesterol solution. The diameter of the T-tube is 1 cm., but the end has previously been reduced in the flame to a diameter of about 1 mm. The T-tube being short has been spliced to permit adequate heating of the solution. The larger flasks marked A must be used during the first dispersion, because the evaporation of the ether causes a lot of foaming. On account of rapid evaporation of ether from the ether-oleate-cholesterol solution, care must be exercised in the prevention of fire.

A variable amount of crystallization will take place, but not sufficient to affect the concentration of cholesterol in the emulsion more than 0.2 or 0.3 per cent. After the emulsion has cooled, it is preferable to subject it to centrifugation. This removes the crystals and apparently also a considerable proportion of the large globules. Such an emulsion will keep for many days or weeks.

Occasionally an emulsion breaks up and crystallizes to a hopeless degree. We have not been able to explain satisfactorily these failures. The emulsion has not as yet been tried on human beings, but sufficient quantity can be given to animals (rabbits) to elevate the blood cholesterol by 50 per cent after intravenous administration.

ELIMINATION OF STANDARDS AND NOMOGRAMS IN THE DETERMINATION OF WHOLE BLOOD SPECIFIC GRAVITY BY A FALLING-DROP TECHNIQUE*

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IN 1924 Barbour and Hamilton¹ described an apparatus for the determination of blood specific gravity by a falling-drop technique. In 1926² they found that in order to obtain sufficient accuracy with the xylene-bromobenzene mixture they used, it was constantly necessary to use aqueous standards and a nomogram. In 1930 Moore and Van Slyke³ tried this method to obtain plasma specific gravity and found it unsatisfactory for routine use because the maintaining of aqueous standards accurate in specific gravity to 1×10^{-4} is so difficult and because constant reference to a nomogram is necessary.

In 1938⁴ I reported a falling-drop method which eliminated the necessity for standards and a nomogram. At that time it was adapted specifically for use with serum and plasma.⁵ Since 1938, however, numerous requests have come for a method of determining the specific gravity of whole blood which would likewise eliminate the use of standards and the nomogram. This determination is desired in order to follow the changes in blood specific gravity during such conditions as shock, dehydration, anemia, and fluid administration, and to study blood volume changes. I have, therefore, adapted the latter method for use with whole blood as described below.

METHOD

It was found that a mixture of methyl salicylate and mineral oil, such as used for the determination of serum or plasma specific gravity, did not permit as large a range as was desirable for use with whole blood.

With the cooperation of the E. H. Sargent & Co. of Chicago, a medium was prepared which consists of a mixture of specially purified petroleum oil and methyl salicylate. It has a specific gravity of 1.0367 25°/25° C., a viscosity of 26.4 cp 25° C., and permits a range of 1.0380 to 1.0720 25°/25° C. This mixture, like the mineral oil-wintergreen mixture for serum and plasma, has a boiling point of over 220° C. and is stable. There is no danger of change in the oil from evaporation such as occurs in the xylene-bromobenzene mixture used in the method devised by Barbour and Hamilton. (A mixture of mineral oil and methyl salicylate prepared four years ago has been tested repeatedly and

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TABLE I
WHOLE BLOOD SPECIFIC GRAVITY
CONVERSION OF FALLING TIME TO SPECIFIC GRAVITY

TEMP. °C.	K	d	TEMP. °C.	K	d
20.0	0.646	1.0390	25.0	0.520	1.0367
20.1	0.644	1.0390	25.1	0.518	1.0367
20.2	0.641	1.0389	25.2	0.516	1.0367
20.3	0.638	1.0389	25.3	0.514	1.0366
20.4	0.636	1.0388	25.4	0.512	1.0365
20.5	0.633	1.0388	25.5	0.510	1.0365
20.6	0.630	1.0387	25.6	0.508	1.0365
20.7	0.628	1.0387	25.7	0.506	1.0364
20.8	0.626	1.0386	25.8	0.503	1.0364
20.9	0.623	1.0386	25.9	0.501	1.0363
21.0	0.620	1.0385	26.0	0.499	1.0363
21.1	0.618	1.0385	26.1	0.497	1.0362
21.2	0.615	1.0384	26.2	0.494	1.0362
21.3	0.612	1.0384	26.3	0.492	1.0361
21.4	0.610	1.0384	26.4	0.490	1.0361
21.5	0.607	1.0383	26.5	0.488	1.0361
21.6	0.604	1.0383	26.6	0.486	1.0360
21.7	0.602	1.0382	26.7	0.484	1.0360
21.8	0.599	1.0382	26.8	0.482	1.0359
21.9	0.596	1.0381	26.9	0.480	1.0359
22.0	0.594	1.0381	27.0	0.478	1.0358
22.1	0.591	1.0380	27.1	0.476	1.0358
22.2	0.588	1.0380	27.2	0.474	1.0357
22.3	0.586	1.0380	27.3	0.472	1.0357
22.4	0.583	1.0379	27.4	0.470	1.0356
22.5	0.580	1.0379	27.5	0.468	1.0356
22.6	0.578	1.0378	27.6	0.466	1.0356
22.7	0.576	1.0378	27.7	0.464	1.0355
22.8	0.573	1.0377	27.8	0.462	1.0355
22.9	0.570	1.0377	27.9	0.460	1.0354
23.0	0.568	1.0376	28.0	0.458	1.0354
23.1	0.566	1.0376	28.1	0.456	1.0353
23.2	0.564	1.0376	28.2	0.454	1.0353
23.3	0.561	1.0375	28.3	0.452	1.0352
23.4	0.558	1.0375	28.4	0.450	1.0352
23.5	0.556	1.0374	28.5	0.449	1.0352
23.6	0.554	1.0374	28.6	0.448	1.0351
23.7	0.552	1.0373	28.7	0.446	1.0351
23.8	0.549	1.0373	28.8	0.444	1.0350
23.9	0.546	1.0372	28.9	0.442	1.0350
24.0	0.544	1.0372	29.0	0.440	1.0349
24.1	0.542	1.0371	29.1	0.438	1.0349
24.2	0.540	1.0371	29.2	0.436	1.0349
24.3	0.537	1.0370	29.3	0.434	1.0348
24.4	0.534	1.0370	29.4	0.433	1.0347
24.5	0.532	1.0370	29.5	0.432	1.0347
24.6	0.530	1.0369	29.6	0.430	1.0346
24.7	0.528	1.0369	29.7	0.428	1.0346
24.8	0.525	1.0368	29.8	0.426	1.0345
24.9	0.522	1.0368	29.9	0.424	1.0345
25.0	0.520	1.0367	30.0	0.422	1.0345

has shown no significant change.) It is this stability which eliminates the necessity for standards by permitting standardization of the oil itself.

The apparatus and technique for determining whole blood specific gravity are identical with those described for serum or plasma protein determination in a previous article.⁴ Briefly, the time taken for a drop of blood to fall a given distance is determined, and the temperature at which this is done is observed; then, by means of a formula or a table, the specific gravity is obtained from the

time and temperature readings. Owing to the high viscosity of the medium used, the distance through which the drop must fall need be only 10 cm. as compared to 30 cm. through the xylene-bromobenzene mixture. This shorter tube allows more uniform temperature throughout the oil and makes transportation of the instrument easy. It can, therefore, be used conveniently in the operating room or at the bedside.

The specific gravity of whole blood is derived from the following formula:

$$\text{Specific Gravity} = K/t + d$$

where K and d are constants for the temperature at which the determination was done and t is the falling time. These constants are shown in Table I. With this formula the mean deviation is less than $\pm 1 \times 10^{-4}$ and the maximum deviation is $\pm 2 \times 10^{-4}$ when compared to specific gravity obtained by pycnometry. For those who require accuracy only to $\pm 1 \times 10^{-3}$ or for rapid estimation in the operating room, Table II is convenient.

SUMMARY

A new oil is reported for use in the determination of whole blood specific gravity by the falling-drop technique previously described by the author.⁴ This oil has the following advantages over the xylene-bromobenzene mixture described by Barbour and Hamilton:

1. Elimination of the use of standard solutions, thus reducing danger of errors which result from changes in these solutions and also making for greater simplicity of technique.
2. Higher boiling point eliminates changes in the oil through evaporation.
3. Higher viscosity permits use of a tube about one-third as long, thus reducing chance of error due to difference in temperature at different levels in the instrument. This also facilitates its use in the operating room or at the bedside because it is more easily transported.
4. It permits substitution of a simple formula for the nomogram.

Using this formula, accuracy may be obtained within $\pm 1 \times 10^{-4}$ in specific gravity. A table is also given which permits direct reading of the specific gravity from the falling time and temperature for those who desire accuracy of only $\pm 1 \times 10^{-3}$.

REFERENCES

1. Barbour, H. G., and Hamilton, W. F.: Blood Specific Gravity: Its Significance and a New Method for Its Determination, *Ann. J. Physiol.* 69: 654, 1924.
2. Barbour, H. G., and Hamilton, W. F.: The Falling Drop Method for Determining Specific Gravity, *J. Biol. Chem.* 69: 625, 1926.
3. Moore, N. S., and Van Slyke, D. D.: The Relationships Between Plasma Specific Gravity, Plasma Protein Content, and Edema in Nephritis, *J. Clin. Investigation* 8: 337, 1930.
4. Kagan, B. M.: A Simple Method for the Estimation of Total Protein Content of Plasma and Serum. I. A Falling Drop Method for the Determination of Specific Gravity, *J. Clin. Investigation* 17: 369, 1938.
5. Kagan, B. M.: A Simple Method for the Estimation of Total Protein Content of Plasma and Serum. II. The Estimation of Total Protein Content of Human Plasma and Serum by the Use of the Falling Drop Method, *J. Clin. Investigation* 17: 373, 1938.

THE ANAEROBIC DRAWING AND SAMPLING OF BLOOD FOR GAS ANALYSIS*

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THE methods generally used in the anaerobic drawing and sampling of blood for blood gas analysis are described by Peters and Van Slyke.¹ The method in most common use is one requiring that blood be drawn from an artery or vein into a vessel containing a layer of mineral oil. Since the oil retards the diffusion of carbon dioxide out of the blood, and the oxygen of the air into it, the oil layer floating on the blood acts as a moderately satisfactory airtight cap. In order to obtain a sample of blood for analysis in a Van Slyke and Neill manometric apparatus, it is necessary that the tip of a stopcock pipette be passed through the oil into the blood. Invariably, a small amount of oil is introduced into the pipette which later causes more or less difficulty in the delivery of the sample into the chamber of the manometric apparatus. A more cautious method, advised by Austin and co-workers,² involves the introduction of drawn blood into a mercury-filled system, the mercury being slowly withdrawn from a receiving vessel into a leveling reservoir as the blood is admitted into the receiving vessel. This most accurate of methods, however, is best carried out by two individuals. If a number of samples are to be drawn within a short time, its use necessitates the possession of a large quantity of mercury, and many pieces of apparatus.

These difficulties are obviated in a procedure which has been used in this laboratory for several hundred analyses of blood oxygen. A 10 c.c. syringe, selected for its smoothly moving, closely fitting plunger, is filled with and then emptied of an anticoagulant, e.g., saturated potassium oxalate. Enough oxalate remains in the syringe to fill the air space in the nipple of the syringe and to form a film on the sides of the barrel. A needle is attached to the syringe, and the sample of blood is drawn. If the sample is of the order of 5 c.c. or more, the volume of anticoagulant remaining in the syringe is an insignificant amount of the sample drawn. After the collection of the sample, the barrel and the plunger of the syringe are held firmly in the right hand while the needle is removed from the nipple of the syringe. A small amount of mercury is then drawn into the syringe from a shallow dish. The syringe is pointed upward, and a drop of blood is forced out through the nipple. A small needle, the lumen of which is no longer patent, is attached tightly onto the nipple of the syringe, providing an airtight cap for the syringe. The mercury may now be shaken back and forth fifteen or twenty times to insure that all the blood is mixed with the anticoagulant.

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In this manner any number of anaerobic samples of blood may be drawn in rapid succession without need of any equipment other than sufficient syringes. If a period of time greater than a few minutes is to elapse before the analyses are made, 20 c.c. syringes may be used instead. In this case, the longer distance separating the tip of the plunger from the upper end of the barrel gives added protection against the possibility that blood gases may diffuse along the inner wall of the barrel. If the sample is to be kept for any length of time, it is advisable to chill the syringe in an ice bath or icebox.

Filling the stopcock pipettes from a syringe is a simple procedure possessing several advantages over older methods. If the syringe is placed flat on a table in such a position that the nipple extends over the edge of the table, the syringe may be held firmly, without danger of admitting air, while the cap is removed from the nipple. A stopcock pipette, to the upper end of which has been attached a 2 cm. length of gum rubber tubing, is attached to the nipple of the syringe. With the stopcock open, the whole assemblage is turned vertically, and the blood is transferred from the syringe to the pipette by gently pressing the head of the plunger against the table top. When the blood level has almost reached the delivery tip of the pipette, the stopcock is closed. The assemblage is again placed horizontally, the stopcock pipette is removed, and the small occluded needle is replaced on the syringe. The layer of blood exposed to the air while passing through the bulb of the pipette is discarded from the tip as the blood level is brought from the top of the pipette down to the upper calibration mark. In this manner the blood actually admitted into the chamber of the gasometric apparatus does not come close to an air interface, and is not subjected to any change in pressure.

The validity of this procedure was checked against the older mercury displacement method by analysis in duplicate of a series of twelve samples of blood in the gasometric apparatus. The average variation in the values for oxygen content was of the same order in each series. The syringe method was found to be simpler and more rapid. It made possible easy chilling and storage of the sample, and permitted the filling of the stopcock pipettes under optimal conditions.

REFERENCES

1. Peters, J. P., and Van Slyke, D. D.: *Quantitative Clinical Chemistry*, Baltimore, 1931, Williams & Wilkins Co., Vol. II.
2. Austin, J. H., Cullen, A. A., Hastings, A. B., McLean, F. C., Peters, J. P., and Van Slyke, D. D.: *Studies of Gas Equilibria in Blood. I. Technique for Collection and Analysis of Blood, and for Its Saturation With Gas Mixtures of Known Composition*, *J. Biol. Chem.* 54: 121, 1922.

MEDICAL ILLUSTRATION

THE CRAYON SAUCE TECHNIQUE FOR MEDICAL ILLUSTRATION*

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FOR the past fifty years a particular procedure has been employed pre-eminently for the illustration of medical subjects. This is known as the crayon sauce or pencil dust technique. From time to time examples of this type of work have come to light which could possibly date the discovery of the procedure before the period previously mentioned. It can certainly be said that Mr. Max Broedel, of the Johns Hopkins School of Medicine, rediscovered the technique and used it extensively in scientific illustration. Prior to this time the pen-and-ink, wash, charcoal, and pencil line techniques had been employed. The latter method was applied particularly for lithographic reproduction, the lithographic grease crayon being used directly on the stone. The pen-and-ink technique was employed to a great extent for the illustration of science during the same period and still is the most popular method of pictorial representation. Wash drawings have been made for many years, but these are rapidly falling into disuse for the illustration of medicine. This is still the most popular method for obtaining drawn tone illustrations in the commercial art field. In the sciences, the necessity of representing on paper a myriad of details called for an exacting and flexible technique, such as that afforded by crayon sauce. The crayon sauce drawing can be developed rapidly in comparison with other techniques.

Knowledge of the Subject to Be Illustrated.—To make the best scientific illustrations one must be able to draw and have a thorough knowledge of the subject to be illustrated. An untrained draftsman who knows biology often can make a better representation of a biologic subject than the trained artist who has little conception of what he is picturing. For example, let us consider the scientific art of medical illustration in the sense of drawing alone. Anatomy is the foundation upon which medical art is built. Without a knowledge of this subject, without this foundation, further work is likely to be done in confusion and fall short of producing a comprehensive illustration.

The average layman has no more knowledge of visceral anatomy than he has of the inside of a house whose doorways he has never entered. Therefore, to draw anatomy one must know anatomy. The artist must be able to draw not only what he sees, but also what he does not see yet knows to exist. For instance, in illustrating a surgical procedure the artist often is handicapped by having to view his subject through a small and poorly lighted incision. The structure

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that he wishes to see is generally covered with blood, superficial fat, mucus, and other obstructions that prevent a clear view. However, if his knowledge of human anatomy is clear and concise, he is able to reconstruct the parts that he does not see but knows to be present. Such knowledge can be obtained only from careful dissection of the cadaver.

The preparation of illustrations for scientific publications is an art in which accuracy and technical detail are absolutely essential. These drawings should have modeling to such an extent that the third dimension is shown with stereoscopic clearness. This profession belongs in a class of its own. The picture produced by the artist in this field must tell a story without a climax. It is a statement of facts as they are, with only a written history of the case to describe the development of the subject portrayed. It is a realistic art in its entirety, an art in which impressionism, cubism, futurism, or any other branch of modernism or ultramodernism has no place whatever.

Types of Medical Drawings.—In explaining or teaching a new operative procedure it is essential that the surgeon have the most important steps of the operation illustrated, because with mere words it is difficult to convey even to the minds of well-trained fellow surgeons the technical details of an operation they have never witnessed.

Practically every operative procedure should be illustrated with black-and-white drawings since color is unnecessary in showing the mechanics of surgery. Once a specimen is removed from the body, however, it often is necessary to make drawings in color. Frequently a diagnosis may depend on the color of a specimen. An accurately colored drawing of such a specimen helps in teaching methods of diagnosis. As a rule, the unusual specimen is the one placed in the hands of the artist.

Drawings play an important part in the teaching of anatomy. The degree of perfection and the importance of a book on anatomy depend largely on the excellence of the illustrations. This is true not only of medical books but of scientific books as a whole.

Training the Medical Artist.—The training of the artist develops his natural ability to observe minute details and to make a mental picture of them. Later he is able to reproduce them on paper because in his mind the picture is clear and complete. In the average person this mental picture is hazy and unfinished; it is completely lacking in the necessary details required by the artist.

In medical illustration it is often customary to make drawings to scale. The artist uses rulers or calipers to determine the correct proportions on paper. As he gains experience he is able to visualize such proportions and combine them on paper in true ratio and proportion to the model. This grasping and reproducing of details, plus the ability to measure with the eye, determine an artist's ability. He must not only make illustrations the exact size as his model, but reductions and enlargements in ratio and proportion to the model or subject he is drawing. For example, he may reduce an entire body to accurate proportions on a sheet of paper that is only 15 inches high. In contrast to this he may enlarge a tiny embryo to accurate dimensions on a piece of paper of the same size.

The following is a description of the materials needed for making medical drawings and their use:

Sketch Paper and Pencils.—In naming the materials used in making medical drawings, let us first differentiate between the paper or board upon which the final drawing is made and the paper for the preliminary sketches. The word *final* is used because such drawings require much detail work and often many corrections and refinements before the drawing is completed. Sketches and outlines are made on an ordinary grade of thin *drawing paper* or a good grade of *typewriting paper* before the final drawing is attempted. After the sketches are prepared and passed on by the biologist or physician who is ordering the

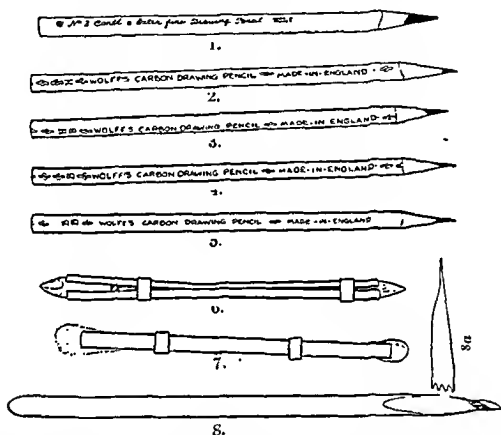


Fig. 1.—Numbers 1, 2, 3, 4, and 5 are pencils, 1 being the Conté or soft pencil from which the pencil dust is obtained; 2, 3, 4, and 5 are Wolff pencils varying in hardness, H being the hardest, and BB the softest. BB and B are used most in developing the crayon sauce drawing. No. 6 is a charcoal holder, in the left-hand side of which is a pointed eraser for removing fine details. The right-hand side is a pointed cork for softening pencil lines. No. 7 is another charcoal holder, in the left-hand side of which is inserted a pad of chamois for softening and blending large areas. The right-hand side contains a blunt cork for softening and blending small areas. No. 8 is the engraving tool or scratcher used for etching out high lights and producing fine, sharp white details. The right-hand side of No. 8a shows how the end of this tool is ground flat. This is the same surface as is presented in No. 8.

drawing, the real work begins. All corrections and refinements should be made in detail on a sheet of thin drawing paper or *sketch paper*, as it is called by medical illustrators. For this purpose a *Conté No. 3 carbon pencil* (Fig. 1, 1) is used; however, a *Wolff BB* (Fig. 1, 5) or a *Wolff B* (Fig. 1, 4) will be found satisfactory. The Conté pencil is soft and transfers easily. The transferring process is essential for making a really fine drawing. The Wolff BB and B pencils are also soft, but neither is as soft as the Conté No. 3. Fig. 1 shows four Wolff pencils of various degrees of hardness or softness. BB is the softest of the four, B is a little harder than BB, HB is harder than B, and H is a comparatively hard pencil. In the preliminary sketches either the BB or the Conté pencils will

be found best and will transfer more readily. All the pencils shown in Fig. 1 are used in making the final drawing on Ross stipple board.

Transfer Process Using an Extra Sheet of Paper.—After the sketch or preparatory drawing is worked up in line, this paper is placed face down on a second sheet of thin paper. By rubbing the back of the first sheet with the fingernails while holding the two sheets firmly together on the drawing board or some other flat surface, a transfer of the soft pencil lines to the second sheet is accomplished. This second sheet will show the drawing in reverse. The parts of the original drawing that were on the right-hand side will now be on the left-hand side on the transfer. In accomplishing this it should be borne in mind that the two sheets must not slip even a fraction of an inch from each other or the transfer will be blurred or out of register. There are various methods of carrying out this procedure. Some artists thumbtack both sheets to a drawing board. If this is done, instead of using the fingernails, which may become sore from rubbing the back of the original drawing, the round or top end of the engraving tool, as seen in Fig. 1, 8, is used. Another method is to clamp together the two sheets of paper with paper clips or clamps. When the drawing to be transferred is no larger than an ordinary sheet of typewriting paper, a hospital chart holder serves excellently to hold the sheets together for the transfer process. The transfer is effected more easily with a few sheets of paper, or a smooth-surfaced piece of cardboard placed under the sheet to which the transfer is to be made. This transfer, which as previously explained is in the reverse order, is called a *negative* (Fig. 2).

Ross Stipple Board.—Before discussing the negative further, let us consider the board or material upon which the final drawing is to be made. This board is known as *Ross stipple board, No. 00-dull* and can be purchased from most of the larger art supply dealers. The surface of this board is prepared in the manufacturing by placing a layer of chalk and sizing on a thin cardboard. This surface is then pressed with a metal plate to obtain the stippled effect of a series of small raised dots which act as teeth to hold the carbon of the drawing pencils. It was necessary for the early medical illustrators to prepare their own stipple board, which they did by applying the chalk and sizing to a flat, finely sandpapered board. However, the material now can be purchased without difficulty.

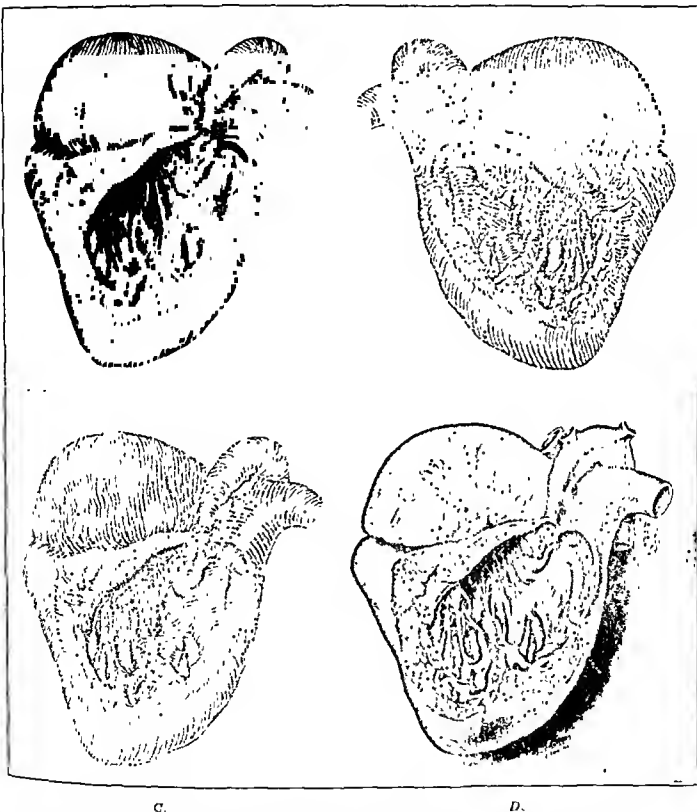
Mounting the Stipple Board.—Before preparing to work up the final drawing on this stipple board, the board should be mounted on heavy cardboard, preferably with rubber cement or by the dry mounting process. A water paste or glue can be used, but it is likely to buckle or curl both the stipple board and the mount. This can be corrected by pasting on the back strips of paper running in the opposite direction from the buckle or bend. As these strips of paper dry they contract and pull the cardboard back to a flat surface.

The negative transfer previously mentioned is placed face down on the mounted stipple board and held in position by thumbtacks, paper clamps, or the hospital chart holder, then rubbed with the fingernails to accomplish a second transfer to the stipple board. Do not rub this second transfer with the round

end of the engraving tool since it may injure the chalk surface of the stipple board that is underneath. This second transfer is in the same order as the original drawing and is called a *positive*. The positive is naturally weak from two transfer processes but serves as a good outline from which to make the finished drawing.

A.

B.



C.

D.

Fig. 2.—Method of making sketch and transfers for the final drawing. This illustration is of a fetal heart. The original drawing was enlarged rather than reduced from the model.

Transfer Process Using One Sheet of Paper.—A second method of making a transfer is as follows:

First, turn the original sketch over face down on a piece of glass under which is an electric light, or hold it up to a window pane. Then with a soft pencil trace the lines that show through the paper. This tracing will be in nega-

tive order. Clamp the sketch, face up, over the mounted stipple board on the chart holder and trace over the original lines of the sketch. The second tracing will cause the lines on the under side of the sketch paper to leave their impression on the stipple board. The transfer processes are necessary because the stipple board does not readily lend itself to making corrections. However, it does make it possible to present detail, texture, and feeling that no other material presents.

The Eraser.—Some illustrators work in the well-known wash method, but this procedure takes longer and requires more skill in handling, and corrections are equally hard to make. Lighter areas in the pencil dust drawing are accomplished by erasing with a *nigrivorine* (No. 3) eraser, half of which has been mounted on a charcoal holder, as seen in the left end of Fig. 1, 6. This eraser must be kept pointed at all times so that detail work may easily be accomplished. There is a similar product called Weldon Roberts shading rubber No. 200.* This serves as well for shading and erasing medical drawings done on Ross stipple board.

Frequently these erasers are a little too soft for a great amount of detail work, even if they are kept pointed. To overcome this difficulty they may be placed in a small cloth bag and thumbtacked outside the window, thereby exposing them to the weather. In three or four months they have hardened sufficiently to work up the detail freely. The *nigrivorine* erasers are not expensive. They are made in France and may be bought at most art supply stores. However, the artist should not depend on this particular eraser alone, but should experiment to find others which will answer his purpose as well. The student should also provide himself with a supply of artgum erasers for cleaning his sketches and the edges of the finished drawing.

Pointed Cork.—In the right-hand end of the charcoal holder of Fig. 1, 6 is placed a pointed cork. This cork can be prepared from almost any cork bottle stopper, but should be chosen from one that is comparatively free of knots or hard areas. The smaller bottle stoppers are best for this purpose. This cork drawing instrument serves similarly to an ordinary paper stump but is superior for softening lines and blending small areas on paper or Ross stipple board.

Chamois and Blunt Cork.—Fig. 1, 7 shows another charcoal holder, the left-hand side of which has been mounted on a piece of chamois wrapped around a small bit of the same material to form a pad. This pad is used to clean up areas of the final drawing when they are found to be too dark. On the right-hand end of this charcoal holder (Fig. 1, 7) is mounted a piece of cork that is round in shape rather than pointed. It is used for softening larger dark areas on the final drawing.

Engraving Tool or Scratcher.—Fig. 1, 8, is undoubtedly one of the most important of the drawing instruments. It is an *engraving tool* made of wood through which passes a flat piece of a very good grade of steel. One side of the wood and steel has been ground flat on an emery wheel, as seen on the right-hand side of Fig. 1, 8a. The point is curved as seen in part 8 of the same

*Manufactured in Newark, N. J., which may be obtained from the F. Weber Company, Philadelphia, Pa.

figure. It is then ground on a fine carborundum flat stone, and polished further on a *hard* Arkansas oil stone. The point must be kept at razor sharpness at all times by means of the Arkansas oil stone, without ever using the emery wheel or carborundum stone a second time. All high lights on the finished drawings are scratched out or etched out of the chalk-surfaced stipple board with this tool.

Use of the Engraving Tool.—Thin lines or pin-point high lights may be made by holding the engraving tool vertically; as the angle to the surface is decreased, a broader line may be drawn. The engraving tool or *scratcher*, as it is called by medical artists, is held between the index and second fingers and guided by the thumb rather than being held as one does a pencil (see Fig. 3). However, use all tools in the manner that is most suitable to you as an individual and work with the kind of tools that you can use best.

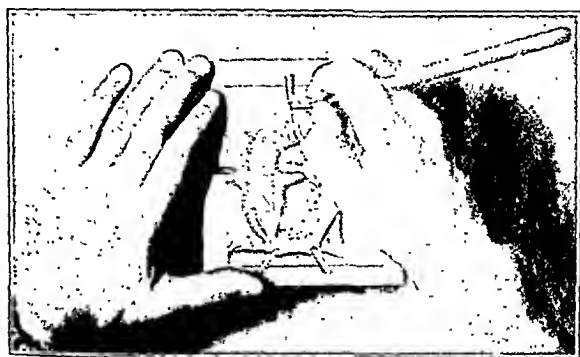


FIG. 3.—The method of holding the engraving tool for scratching out high lights in drawings on Ross stipple board. This tool must be kept at razor sharpness, otherwise harsh ragged lines will result.

Charles Dana Gibson stated in one of his lectures that many art students ask him what particular make of pen point he uses in making his pen-and-ink drawings. His answer was that he uses any kind he finds suitable to draw with. After all, it is the artist who uses the instrument rather than the instrument that makes the drawing. However, certain instruments are better for specific purposes.

Unless the scratcher is kept sharp it not only becomes cumbersome to work with, but invariably makes broken, irregular high lights or white lines that reduce the quality of the drawing. The further use of these tools will be described fully when we actually begin the making of a scientific illustration.

Pencil Dust or Crayon Sauce.—The final drawing is worked up on stipple board with carbon pencils or crayon sauce that is applied with brushes. Crayon sauce may be prepared by sandpapering the center or so-called lead of the *Camé* No. 3 pencil. This pencil serves best for this sauce since it is of the right

softness. Harder pencils give a dust that does not grip or stick to the stipple board quite so readily. This dust or sauee may be saved in a small box, such as those in which the more expensive cigarettes are packed. An ideal way to collect it is to mount an ordinary sandpaper pad that is purchased in art stores (for sharpening charecoal) on an angle and to a side in one of these boxes. When the Conté pencil is pointed on the pad the dust slides from the pad into the box. In this way the artist kills two birds with one stone; he points his pencil and saves the dust which is later applied to the drawing. It might be well to bear in mind that no other pencils but the Conté No. 3 should be pointed in this box, as

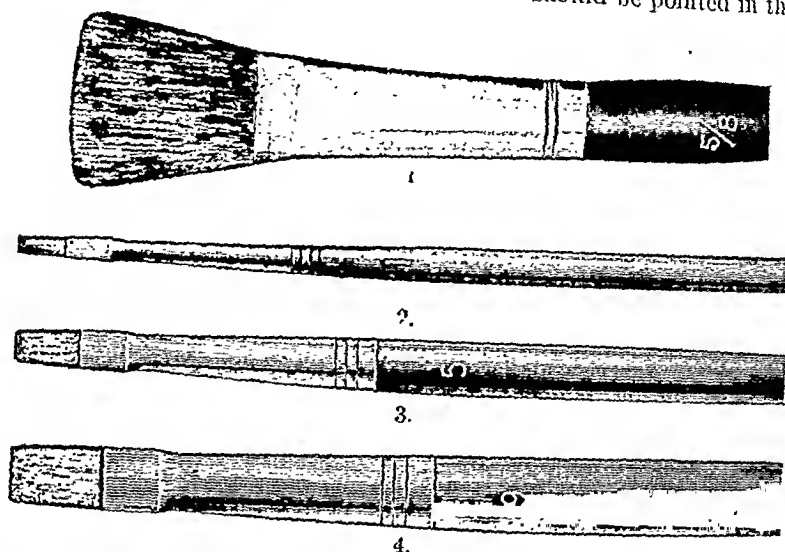


Fig. 4.—Brushes used for the pencil dust or crayon sauee technique. No. 1 is a large camel's hair or sable blending brush. Nos. 2, 3, and 4 are red or black flat sable brushes for applying tones. The numbers written on the handles of the brushes denote their sizes. This illustration is reproduced the exact size of the original brushes. (From the catalog of F. Weber Co.)

only the soft Conté No. 3 dust is suitable for applying with the brushes. Under no conditions should an ordinary lead pencil be sharpened on the pad, because carbon pencil lines will not stick to the surface of the paper over ordinary pencil lines but will slide smoothly over them and leave no mark at all. A mixture of these dusts will only lead to difficulties. Should the artist not care to save this Conté dust in the manner described, he will be able to purchase it already prepared under the name of crayon sauee. However, he will find that the numerous necessary sharpenings of his Conté pencil will provide an ample supply.

Brushes.—There are four brushes used for applying this Conté dust. Fig. 4, 1 shows a fair-sized sable blending brush. This is suitable for applying an even tone over the entire surface of the paper. Darker tones may be put in with one of the other three brushes seen in Fig. 4, 2, 3, and 4. The smaller brush is used for fine work and the others for covering larger masses. The brush shown in Fig. 4, 3 will be the one that is used most.

Obtaining Dark Tones.—When the drawing is practically finished, the student will find that there are areas which must be made darker when the stipple

board will not take a tone of sufficient blackness. This can be accomplished by pouring (not atomizing) alcohol on the drawing and then draining it off at a corner back into the bottle. After the alcohol has drained off sufficiently to lose its glossy, wet appearance, more pencil dust can be applied with the brush. The stipple board thus treated with alcohol permits darker tones. However, this should not be done until the drawing is practically finished, as any erasing thereafter becomes more difficult. The alcohol has a tendency to harden the chalk surface of the stipple board. Never pour alcohol or fixative on a charcoal drawing. It is necessary to apply the alcohol or fixative to charcoal drawings with an atomizer.

After this alcohol treatment there may be points that still need to be made darker. This can be accomplished with pen and India ink; however, bear in mind that the less it becomes necessary to apply ink, the better the drawing is likely to be. Work continuously with pencils, crayon sauce, and brushes until you can see no other points to improve, then apply the ink to points that you find impossible to make darker with the pencil or crayon sauce. Any number of applications of alcohol may be applied without harming the drawing, except for erasing. When entirely finished, the drawing may be fixed with ordinary alcohol fixative (for charcoal drawings) that has been diluted with three parts of alcohol.

Summary of Materials.—The following is a summary of the materials used in making drawings in pencil dust on Ross stipple board:

An ample supply of thin drawing paper or a good grade of typewriting paper. Some of the sheets should be twice typewriting paper size for large drawings.

Ross stipple board No. 00 dull.

A supply of heavy cardboard for mounting, at least 14 ply.

Water paste, rubber cement, or dry mounting process for mounting.

One engraving tool or scratcher.

Conté No. 3 pencils.

Crayon sauce.

Wolff pencils—BB, B, HB, and H.

Two charcoal holders.

Cork for stumps.

A piece of chamois.

Three flat red or black sable brushes, $\frac{1}{8}$, $\frac{1}{4}$, and $\frac{1}{2}$ inch wide.

One blending brush about 1 inch wide.

Nigrivorine erasers.

Artgum erasers.

THE PENCIL DUST TECHNIQUE

As the antiquated axiom goes, "Rome wasn't built in a day," so it is with developing the ability to draw. We do not acquire perfection in the production of scientific illustrations overnight. For this reason, the study of the pencil dust technique should be started with comparatively simple subjects, such as a few Malaga grapes, a rubber tube or ball. Before accepting these as simple

subjects let us first study them carefully. There are high lights, reflected lights, and shadows to be considered. Grapes are semitransparent or translucent. This effect of transmitted light is not easy to obtain on paper, therefore one's first drawing should be undertaken with great care and a determination to produce a satisfactory illustration.



Fig. 5.—A pencil dust drawing of two balls or spheres.



Fig. 6.—A pencil dust sketch of a white rubber tube. Such practice drawings are made in an effort to learn to produce the effect of the third dimension.

When a drawing is copied from a reproduction, it should be enlarged many times, at least to a convenient working size. When drawings in tone are copied, they should be copied from the original drawings if possible, though it is far

better to work from the actual subject. For this reason, simple subjects easy to obtain are mentioned. Where it is not possible to obtain the same subject, a similar one will serve. For instance, white marbles or large clear glass beads could take the place of grapes. The drawing of grapes, marbles, or beads develops a dexterity in drawing the cysts that are so often present in pathologic specimens. While some of these subjects will be quite simple and seem to possess little bearing on scientific illustration, they have been chosen for a definite purpose. The training received in drawing them will be useful when actual scientific subjects present themselves.

Make a careful line sketch of the model with a Conté No. 3 or Wolff BB pencil. This sketch may be the same size as the subject. It is best to try to make this, as well as the subsequent sketches, in line rather than graded tones, because this helps to develop a feeling for lines and how their placing, running directions, and curves can determine various ideas of modeling and shading. This rule should be followed in making all scientific drawings for transfer. Such

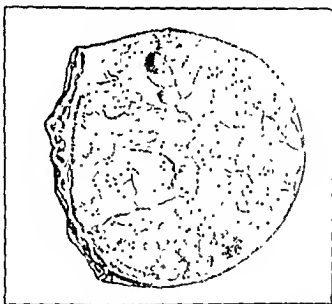


Fig. 7.—A small drawing of an ovarian cyst. This type of drawing shows one of the first subjects a student should undertake in developing a technique for illustrating pathologic specimens. Many principles he should have learned from drawing spheres, depressions, raised areas, and grapes apply to the making of such an illustration.

practice is invaluable when it becomes necessary to make pen-and-ink drawings which are generally done in line only. There is a dry brush and India ink technique used quite often by commercial artists, but I believe that at present it is not well suited to scientific illustration. However, there is no reason why some excellent draftsman should not be able to develop a brush and ink technique suitable for this purpose.

Transferring.—After this line sketch is well developed, transfer it as previously described. It is this transfer which is to be worked into the final drawing.

Developing the Pencil Dust Drawing.—First, carefully outline the subject with a Wolff BB pencil. Do not make these outlines too heavy, as it must be remembered that they are eventually to be blended into the tones of the body of the drawing. A general tone now may be applied to the entire surface of the paper with the blending brush. Dip the brush in your box of crayon sauce

and then wipe it off on the chamois pad for that purpose. Lightly brush over the entire surface of the drawing, thus giving it a general tone of about the shade you consider the greater part of the original to be. Using this as a key, pick out the high lights with the nigrivorine eraser and apply the darker tones with the flat sable brushes.

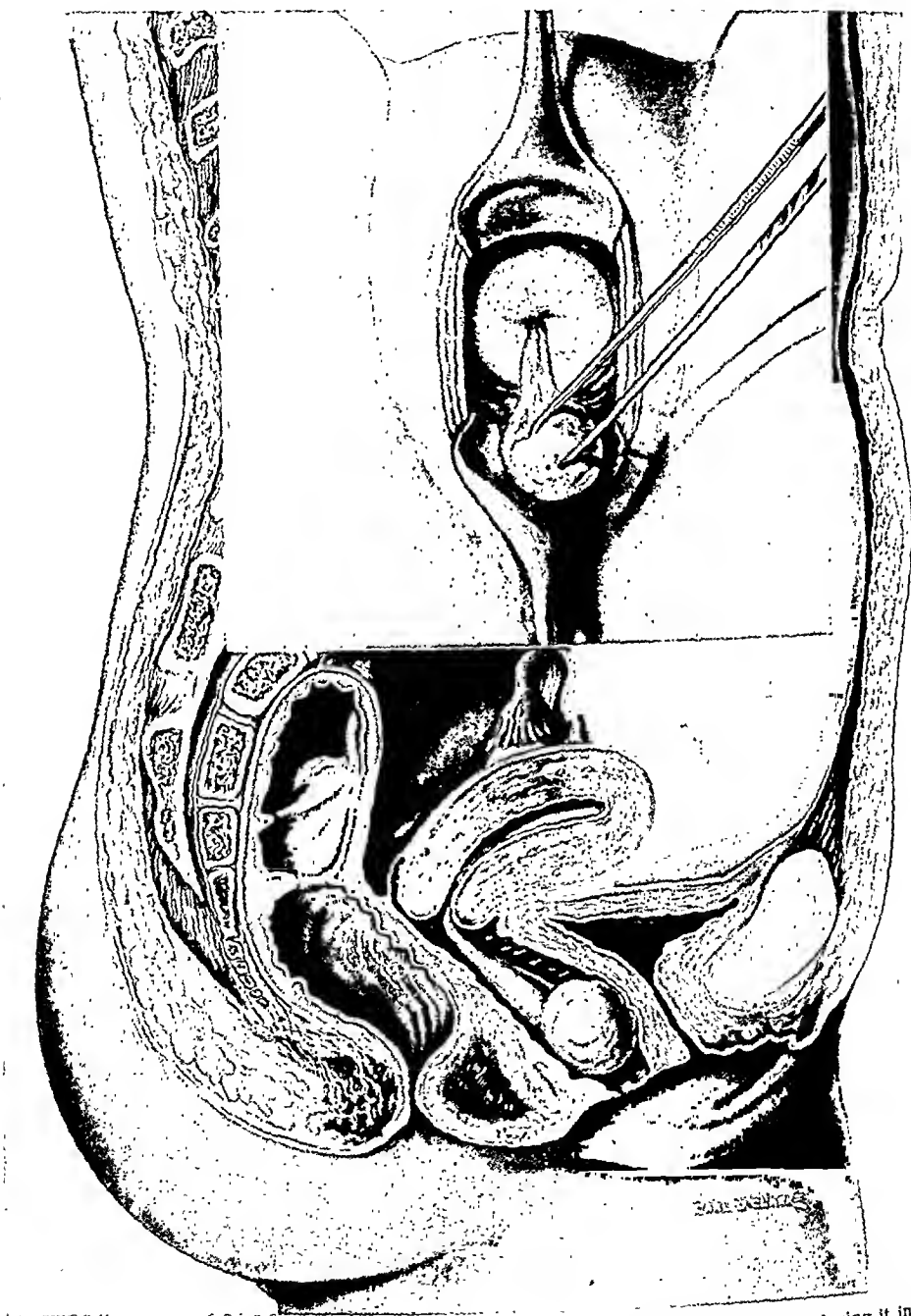


Fig. 8.—Two views of a single pathologic specimen showing the method of placing it in its natural surrounding structures as a means of orientation. Many principles learned in the simpler drawings make it possible to produce a more complicated drawing as illustrated above.

No hard and fast rules are set down for the remainder of the work of developing the drawing. The student should work in the way which suits him best. The following directions are no more than suggestions of how to use the materials for the pencil dust technique. Some students prefer to pick out the high lights and lighten the areas that are lighter than the general tone. Others start right in with the brushes to obtain the darker gradations. Either method is acceptable.

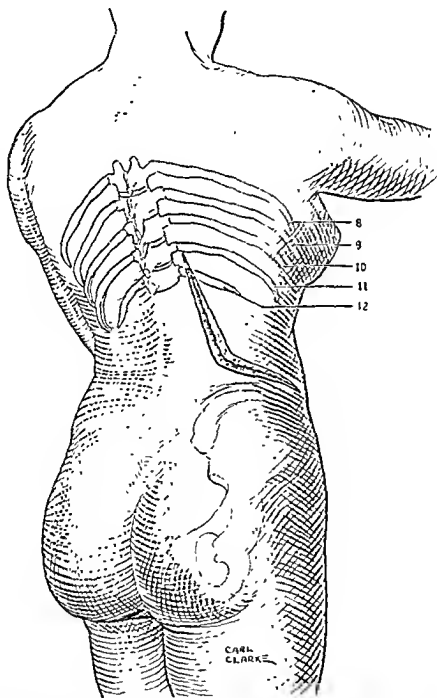


Fig. 9.—In illustrating a surgical procedure the operative technique should be developed step by step. For example, this illustration shows the first step in an operation for the treatment of nephroptosis. The caption published under this figure read as follows. "A modified hockey stick incision. The intercostal muscle is exposed in the upper end of the wound. An adequate exposure is obtained with a maximum of consideration to anatomic details." (From the Bulletin of the School of Medicine, University of Maryland.)

Working From Light to Dark.—Find the definite high lights, which can be little more than points. Erase these from the general tone with the pointed eraser. Larger areas may be wiped out with the mounted chamois. If these areas are to be made still lighter, they may be erased by lightly touching or gliding the pointed eraser over the surface of the paper. Definite points of light may be etched or scratched out with the engraving tool. However, this

should not be done until the drawing is well modeled with the brush and eraser. If the engraving tool is used too soon, the drawing may lack depth of modeling. These are terms employed to express the illusion of the third dimension on a flat surface such as drawing paper. After the drawing is well modeled, the engraving tool is used to etch out minute points of light and to lighten small

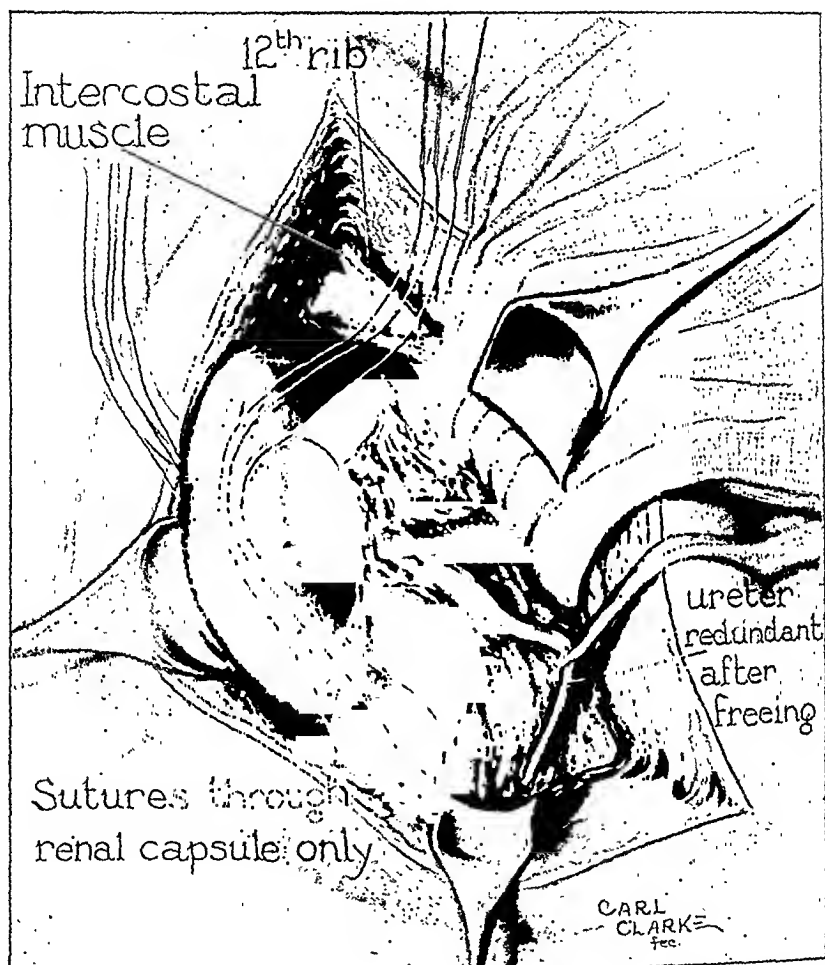


Fig. 10.—Another step in the development of the same surgical procedure. The caption under this figure read as follows: "A method of placing sutures in the renal capsule. These sutures may vary in position as desired. The pedicle and ureter are freed of connective tissue. The anterior surface of the kidney is shown and the ureter is pulled medially." (From the Bulletin of the School of Medicine, University of Maryland.)

areas by gliding the instrument over the paper. If this tool is of razor sharpness and handled lightly, it will remove only the tops of the tiny raised areas of the stipple board and create an additional tone that will improve the drawing as a whole. The best illustrations have a full range of tone from the lightest light to the darkest dark, with numerous graded tones between.

A brush of medium size that has been dipped in the crayon sauce may be used to blend carefully and lightly in the shades of tones on the shadow side of

the drawing. Bear in mind that space must be saved for the reflected lights and that the tone is darkest next to these reflected lights. After these tones are shaded in to the best of your ability, take the pointed cork and soften the outlines that were drawn in the beginning. Be careful not to let the tone which is spread by the cork come too far over into the reflected lights, or the lighter areas, as a wide line. Should this happen the overlapping tone may be removed by the pointed eraser.



Fig. 11.—In this step of the procedure, the free ends of the capsular sutures are brought out through the intercostal muscle above the twelfth rib before the kidney is drawn into place. (From the Bulletin of the School of Medicine, University of Maryland.)

Use of Drawing Instruments.—As the drawing develops use the instrument which suggests itself. When a point or area is too dark, use the eraser, chamois, or engraving tool to lighten it. When it is too light, use the cork, brushes, or pencils to darken it. It is a constant alternating from one instrument to another and a careful working with them that eventually produces a finished drawing.

Study the high lights, reflected lights, shadows, and translucencies of the subject and try to duplicate them in the drawing. Translucency may be difficult to obtain but by constant effort it can be accomplished.

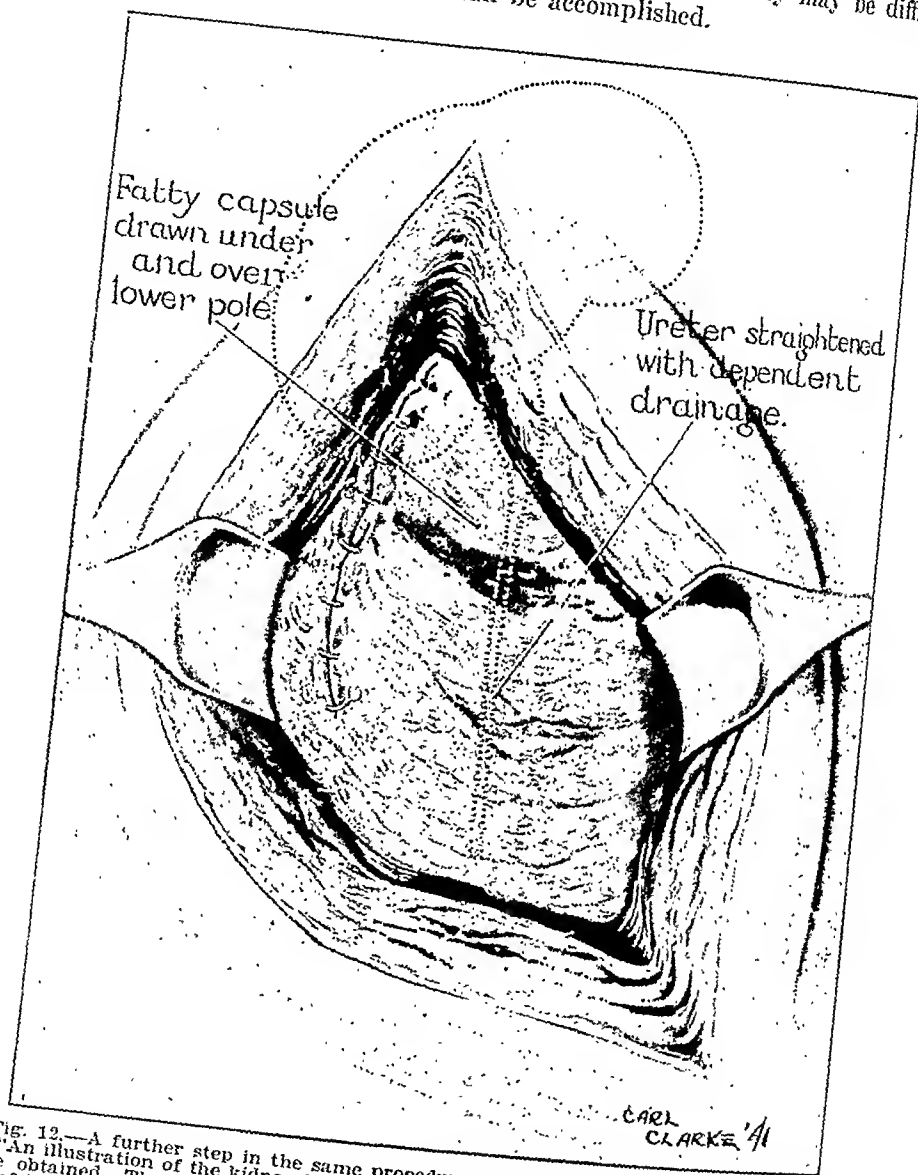


FIG. 12.—A further step in the same procedure. The caption under this figure read as follows: "An illustration of the kidney in the desired position. Overcorrection and dependent drainage are obtained. The fatty capsule supplies secondary support." (From the Bulletin of the School of Medicine, University of Maryland.)

You will find that the spotted effects so often seen on fresh pathologic specimens can be produced by just touching the surface of the stipple board with the pointed eraser and cork. As you work, such ideas of obtaining different effects suggest themselves.

Developing the Darks.—After you have worked the drawing to a point where the stipple board will not take any darker tones, flow the alcohol over the mounted drawing and allow it to drain back into the bottle from a corner. Be sure that the entire surface of the paper is covered with alcohol, otherwise a line

may form between the covered and uncovered surfaces. As soon as all indications of wetness have disappeared, a second coat of crayon sauce may be applied where necessary to obtain darker tones.

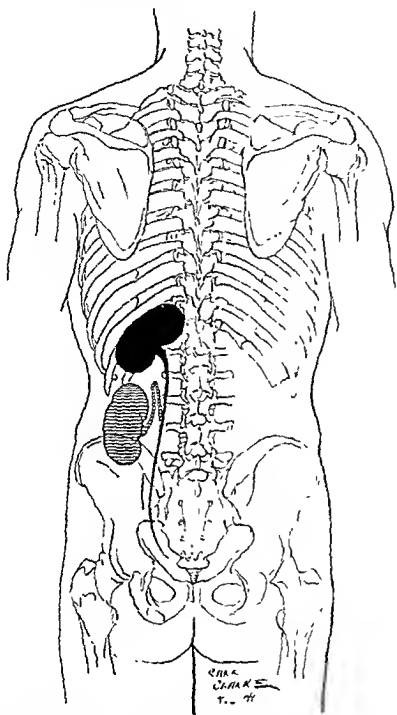


Fig 13.—The final illustration in the surgical technique previously described. The caption under this figure read as follows: "A diagrammatic posterior view of a hypothetical case and the degree of correction possible." (From the Bulletin of the School of Medicine, University of Maryland.)

If there are areas that are still to be made darker, this may be accomplished by passing the point of the Wolff BB or Conté pencil lightly over them. In such a case do not bear hard enough on the pencil to press into the paper or to leave a microscopic mound of carbon at the end of a pencil line. It is better to go over the area again and again until the desired tone is produced. The final dark touches to the drawing may be done with pen and India ink. This should be applied sparingly, otherwise it may be conspicuous to the point of affecting the quality of the drawing.

Fixing Pencil Dust Drawings.—After you have worked on the drawing until nothing further suggests itself, it should be made permanent with a weak fixative of one part fixative to three parts of alcohol.

Fixative of normal strength can be made by dissolving 2 ounces of gum mastic in 8 ounces of alcohol. For pencil dust drawings this should be diluted to a point where it will leave no gloss on the stipple board after it is dry. This can be done by adding three parts of alcohol to one part of fixative.

Shellac fixative can be made as follows: Dissolve $\frac{1}{4}$ pound of dry white shellac in a quart of alcohol. Shake well every few days until the shellac is completely dissolved, then let it settle for ten days and pour off the clear yellow liquid above the shellac that has settled to the bottom of the container. Be careful not to allow any of the sediment to mix with the clear solution. For pencil dust drawings this should be diluted with about six parts of alcohol.

It is best for the fixative to be no stronger than necessary to keep the pencil dust from smudging or coming off the board. While drawings can be worked on after fixing, it is better to develop them as far as possible before fixing.

REFERENCE

Clarke, C. D.: *Illustration, Its Technique and Application to the Sciences*, Baltimore, 1940, John D. Lucas Co.

DEPARTMENT OF ABSTRACTS AND REVIEWS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

MENINGITIS, Pneumococcal and Influenzal, Treatment of, With Sulfapyridine and Its Sodium Salt, Neal, J. B., Appelbaum, E., and Jackson, N. W. J. A. M. A. 115: 2055, 1940.

The authors present a series of 30 cases of pneumococcal meningitis with 10 recoveries and 29 cases of meningitis due to *H. influenzae* with 14 recoveries treated with sulfapyridine, its sodium salt, or both. A specific serum was usually employed as well. However, they believe, in view of previous experience, that the lower case fatality in both forms of meningitis is attributable largely to this form of chemotherapy.

SMEGMA BACILLUS, Differentiation of Tubercle and, Bent, M. J., and West, H. D. Am. Rev. Tuberc. 42: 815, 1940.

The disappearance of color in cultures of smegma bacilli grown on Petraguani's and on Petroff's media containing malachite green can be used to differentiate these organisms from tubercle bacilli which are not capable of producing the change in these culture media.

The color change results from a tautomeric shift of the dye molecule to the colorless color base of malachite green because of increased alkalinity. The increased alkalinity is due to the greater production of ammonia by the smegma bacilli.

Autolysis of smegma bacilli with the production of ammonia is probably an important factor.

BONE, Giant Cell Tumor of, Jaffe, H. L., Lichtenstein, L., and Partis, R. B. Arch. Path. 30: 993, 1940.

A conception of the giant cell tumor of bone has been developed which can be briefly summarized as follows: The giant cell tumor of bone is not as common or as miscellaneous a lesion as it has generally been supposed to be. Cases of medullary fibroma or osteofibroma of medullary xanthofibroma or xanthogranuloma, and of certain peculiar forms of medullary chondroma, for instance, are sometimes misclassified under the head of giant cell tumors—notably as representing supposed variants of the latter. The authors deprecate this tendency and hold in particular that cases classed under the head of "osteitis fibrosa or spindle cell variant," "xanthic variant" or "chondromatous variant" of giant cell tumor actually represent misclassified cases of these other, independent conditions. They have frequently made such misclassifications. Furthermore, they feel that the resemblance between the so-called "brown tumors" of hyperparathyroidism and certain epulides, on the one hand, and genuine giant cell tumors, on the other, is only superficial and is not based on any pathogenetic relationship.

The authors interpret the giant cell tumor of bone as a neoplasm of a definite kind, arising apparently from the undifferentiated supporting connective tissue of the marrow and clearly delimitable on the basis of its cytologic details. In essence, it is composed of a more or less vascularized network of spindle-shaped or ovoid stromal cells and multinuclear giant cells in certain particular proportions and arrangements. The tumor shows but little collagenous differentiation of its stroma and almost no evidence of ossification. However, there are differences between individual giant cell tumors, particularly in respect to aggressiveness, based mainly on differences in stromal cell detail. On the basis of these differences, we have found it expedient to subclassify giant cell tumors into three grades.

Those which are classed under grade I are the least aggressive ones, in which the stromal cells, though abundant, are not compacted and do not exhibit any appreciable atypism. The tumors of grade II manifest cytologically a very compact cellular stroma, showing definite evidences of atypism, tend strongly toward recurrence, and in some cases eventually undergo malignant transformation. Those of grade III represent the small group which possess a sarcomatous type of stroma, are frankly malignant and metastasize. The malignant giant cell tumors are characterized microscopically by the presence of abundant, compacted stromal cells appearing in whorled arrangement and uniformly showing signs of atypism.

From the clinical point of view this study has shown that a genuine giant cell tumor is not likely to develop in any person below the age of 20. In connection with the interpretation of the roentgenographic findings, it is pointed out that, despite the common opinion, there is no such thing as a "typical" or a "characteristic" roentgen picture of a giant cell tumor. It is maintained further that "blind" irradiation based on an unverified roentgenographic diagnosis is usually not justifiable and that a biopsy should be performed on a bone lesion suspected of being a giant cell tumor to establish the diagnosis definitely before therapy is undertaken. The treatment of a giant cell tumor of bone should be guided by the idea that this lesion (considered independently of its supposed variants) is, on the whole, a more serious one than it has usually been supposed.

SULFATHIAZOLE in Blood and Urine, Sunderman, F. W., and Pepper, D. S. Am. J. Med. Sc. 200: 790, 1940.

Applying methods for the analysis of sulfanilamide in whole blood to the measurement of sulfathiazole, the authors have found that recovery of sulfathiazole in whole blood averages approximately 86 per cent of the theoretical. This diminution in the recovery was shown to occur during protein precipitation. Employing serum instead of whole blood, loss of sulfathiazole is considerably less, averaging only about 3 per cent. For this reason, the authors feel that analysis for sulfathiazole should be made in serum instead of whole blood. A procedure for analysis in serum is suggested in which an arbitrary correction for the loss is made in the final calculation.

Since both sulfathiazole and its acetyl derivative are about twice as soluble in urine of pH 7.6 as in urine of pH 5.6, it may be inferred that when crystalline concentrations owing to sulfathiazole therapy threaten, an effort should be made to keep the urine alkaline and to secure a large urinary volume.

The method for serum follows: Two milliliters of serum obtained from centrifuged clotted blood are run into a 25 ml. volumetric flask containing 10 ml. of 8 per cent trichloroacetic acid. After shaking thoroughly to keep the precipitate finally divided, the solution is diluted to the mark with distilled water. The contents are mixed and allowed to stand for twenty minutes; then filtered through Whatman No. 44 paper to obtain a clear, colorless filtrate. Ten milliliters of the filtrate are diazotized at room temperature with 1 ml. of 0.1 per cent freshly made solution of sodium nitrite. The solution, after standing for three minutes, is treated with 1 ml. of 0.5 per cent solution of ammonium sulfamate. After mixing thoroughly, 5 ml. of dimethyl-alpha-naphthylamine coupling reagent are added; the tube is stoppered, inverted once, and allowed to stand for ten minutes.

Comparisons of color development are made by means of a colorimeter (or photoelectric cell device), utilizing sulfathiazole standards of appropriate concentrations carried through the same procedure as with the unknown. It is important that the solutions be well shaken before placing them in the colorimeter cups to eliminate small gas bubbles that may be present. (The gas is presumably nitrogen formed by the destruction of HNO_2 with $\text{NH}_2\text{SO}_2\text{NH}_2$.) The authors employ a colorimeter (Bausch & Lomb) having an attachable lamp. The eyepiece of the colorimeter is fitted with a No. 74 Wratten filter. Standards of appropriate concentrations are made from a stock solution of sulfathiazole containing 200 mg. per liter. It is convenient to have prepared standards with concentrations of sulfathiazole of 0.2, 0.4, and 0.8 mg. per 100 ml., respectively.

Calculation:

Reading of Standard

Reading of Unknown \times mg. of Sulfathiazole in Standard \times Dilution factor \times Correction factor = mg. per 100 ml. Correction factor = 1.03.

Recovery of sulfathiazole from protein-free urine is practically quantitative. The procedure for serum may be applied to urine determinations disregarding the correction factor. It is preferable that appropriate dilutions of urine be made so that sulfathiazole concentration in the diluted urine is between 5 and 30 mg. per 100 ml.

MENINGITIS, Pneumococcic, Treatment of, Rhoads, P. S., Hoyne, A. L., Levin, B., Horswell, R. G., Reals, W. H., and Fox, W. W. J. A. M. A. 115: 917, 1940.

In a series of 22 consecutive cases of pneumococcic meningitis treated by the authors with the combined use of sulfanilamide or sulfapyridine (and its sodium salt) and specific antipneumococcus rabbit serum there were 7 recoveries.

In the period covering these cases, more patients were admitted to the Cook County Hospital with pneumococcic meningitis than with meningococcic meningitis. The old teaching that purulent meningitis should be treated as meningococcic meningitis until proved otherwise is entirely outmoded. A vigorous search for the etiologic agent must be started at once and pursued until the organism is determined. Pending that time, sulfapyridine by mouth or its sodium salt intravenously should be given in large doses, unless hemolytic streptococci are strongly suspected, in which case sulfanilamide may be the better chemotherapeutic agent.

The best prognosis in cases of meningococcic meningitis is in so-called primary cases, i.e., those unassociated with other demonstrable pneumococcic lesions. The worst prognosis is in those cases associated with pneumococcic endocarditis and/or extensive pneumonia.

In addition to vigorous chemotherapy and serum therapy, surgical drainage of focal lesions, care to maintain an adequate fluid and caloric intake, and occasionally blood transfusions, are of the utmost importance.

RECTUM, Prognosis in Carcinoma of, Broder, A. C., Buie, L. A., and Laird, D. R. J. A. M. A. 115: 1066, 1940.

A total of 432 resected specimens of carcinoma of the rectum were graded according to Broder's method and grouped according to Duke's method of classification. The distributions of grade and class were studied in comparison with other investigations and also in relationship to survival after operation. On the basis of the entire study, certain conclusions were reached:

1. The presence or absence of mucus loses importance as a guide to prognosis if histologic grading is done by Broder's method.
2. Tumors of higher grades are more rapid in growth, and their metastases cause the death of the patient earlier than those of lower grades.
3. The classification of the lesion according to the method of Duke is also correlated with postoperative life; the higher the class, the less the percentage survival.
4. A combination of Broder's grading and Duke's classification yields a prognosis of survival more accurate than either method taken separately.

HYPERTENSION and the Surgical Kidney, Braasch, W. F., Walters, W., and Hammer, H. J. J. A. M. A. 115: 1837, 1940.

One hundred and fifty approximately consecutive nephrectomies were studied. These cases were representative of severe unilateral renal damage.

Hypertension was not a common finding in the clinical picture in this group.

Vascular changes, which have been considered to be causative factors in the production of hypertension, were present in a high percentage of the cases.

Elevation of blood pressure readings in these cases was not the rule, even in chronic pyelonephritis. Nephrectomy was not followed by appreciable reduction in blood pressure readings before operation.

The exact etiologic factor in renal (ischemic) hypertension is as yet unknown. The pathologic and anatomic elements seem less important than an as yet unknown physiologic element.

BIOPSY, Testicular, Its Value in Male Sterility, Charny, C. W. J. A. M. A. 117: 1429, 1940.

The testis, with the serotal skin taut, is held in the operator's left hand. After application of an antiseptic, a portion of the skin overlying the anterior surface of the testis is infiltrated with 1 per cent procaine hydrochloride. An incision about 1.5 cm. long is made through the skin and carefully carried through all the fascial layers until the parietal layer of the tunica vaginalis is opened. This is signalized by the escape of serous fluid and the appearance of the glistening visceral layer of the tunica vaginalis. The latter and the tunica albuginea are nicked with a small scalpel. Gentle pressure on the testis now serves to extrude a small bead of tissue, which is cut off with a curved iridectomy scissors. The testicular incision need not be sutured. The skin is closed with two interrupted catgut sutures and a collodion dressing is applied. The patient may return to his former duties the following day, wearing a suspensory for a day or two. The specimen of tissue is preserved in Bouin's solution, mounted in paraffin for section, and stained by hematoxylin eosin.

Testicular biopsy is a simple, innocuous procedure, as shown by its application in 40 infertile patients without a single mishap.

Testicular biopsy is not only a definite diagnostic aid in differentiating between obstructive and nonobstructive semen defects but serves a prognostic function as well.

When performed before and after treatment, testicular biopsy yields the most direct evidence of the efficiency of the therapeutic agent employed.

PNEUMOCOCCIC INFECTION, Changes in the Number of Circulating Leucocytes in Relation to Spontaneous Recovery From, Fleisher, M. S., and Rich, G. T. Arch. Path. 30: 843, 1940.

The change (relative increase or decrease) of the total number of leucocytes and of the number of neutrophils per cubic millimeter of the circulating blood of the guinea pig twenty-four hours after infection with type I pneumococci bears a positive relationship to the spontaneous death or recovery of the animal. Even at six hours after infection this relationship is suggested or is evident. This positive relationship can be observed when death or recovery is postponed for more than twenty-four hours subsequent to six-hour or twenty-four-hour counts. Study of the response of animals to a nonspecific irritant suggests the possibility that this early reaction of the leucocytes is at least in part an inherent physiologic adaptation of the animal.

BLOOD VOLUME and Extracellular Fluid Volume in Infants and Children, Robinow, M., and Hamilton, W. F. Am. J. Dis. Child. 60: 827, 1940.

Blood volumes of newborn infants averaged 98.3 \pm 8.7 c.c. per kilogram of body weight. The relative plasma volumes varied inversely with the hematocrit reading. A tentative explanation for this phenomenon is given.

"Extracellular fluid volumes" were calculated from the dilution of intravenously injected sodium sulfocyanate. These volumes increased with age if calculated on the basis of body surface but decreased if calculated on the basis of body weight.

Extracellular fluid volumes were found to be large in nephritic edema and in malnutrition. Low values were found in obesity and in dehydration.

Histamine shock in dogs did not change the concentration of sulfocyanate in plasma. The fluid of edema in acute nephritis is apparently entirely extracellular.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor,
Dr. Warren T. Vaughan, 201 West Franklin Street, Richmond, Va.

Diagnosis and Treatment of Menstrual Disorders and Sterility*

THIS book is a well written, thorough summary of endocrine functions in gynecology which is easy to read and interesting. It is written more from the clinical than from the didactic standpoint, and thus is of practical benefit to the practitioner. The authors take up the entire field of menstrual disorders very thoroughly as to diagnosis and treatment, and include sufficient aspects of anatomy, etiology, and pathology to present a complete picture. There is also a short but comprehensive summary of the normal physiology of the menstrual cycle. The subject of sterility is thoroughly taken up as to etiology, diagnostic study, and treatment.

The book is well illustrated and contains tables illustrating differential diagnosis. There is a good bibliography at the end of each chapter and a well-prepared index.

Synopsis of Materia Medica, Toxicology, and Pharmacology†

EXCEPT for the dimensions of the volume this can hardly be considered a synopsis. With over 600 pages printed in small type, it is a very comprehensive pharmacology. The book is small, and therefore, might be termed a handbook. Otherwise it is an excellent reference work on pharmacology.

It takes up the subdivisions of the subject in logical order, starting with basic principles, then materia medica, and prescription writing, followed by toxicology.

To the reviewer this seems to be a very logical approach, and one which is usually not followed since most of these subjects appear in an appendix at the end of a book. Obviously, these are phases that could well be mastered by the student before starting with the actual basic considerations of the action of drugs.

In the body of the book, drugs are grouped in accordance with the localization of their action, whether it be in the skin, central nervous system, the peripheral nervous system, muscle, the urinary tract, etc. Final chapters deal with vitamins, serums, vaccines, hormones. The sulfonamide compounds are grouped with several other drugs in a final miscellaneous chapter.

The American College of Physicians‡

THIS volume is unique in that it presents a complete and accurate history of one of our outstanding medical organizations from its birth in 1915 to the present time. The editor,

*Diagnosis and Treatment of Menstrual Disorders and Sterility. By Charles Mazer, M.D., F.A.C.S., Assistant Professor of Gynecology and Obstetrics, Graduate School of Medicine, University of Pennsylvania; Gynecologist to the Mount Sinai Hospital, New York City. 1940. 108 pages. \$6.50. Paul B. Hoeber, Inc. London, 1941.

†Synopsis of Materia Medica, Toxicology, and Pharmacology. For Students and Practitioners. By Ramon Davison, B.A., M.Sc., Ph.D., M.B., Assistant Professor of Medicine, University of Arkansas, Little Rock. Cloth, pages 600. The C. V. Mosby Company, St. Louis, 1940.

‡The American College of Physicians. Its First Quarter Century. Historian William C. C. Morgan, M.D., LL.D., Sc.D., M.A.C.P., Fellow of the American College of Physicians, Secretary-General (1929-32), Secretary-General (1932-37), of the American College of Physicians; Professor of Medicine, Georgetown University School of Medicine, Washington, D.C. 1940. 275 pages. \$2.00. American Medical Association, Chicago, Ill.

Doctor William Gerry Morgan, is peculiarly qualified for the assignment of chief historian. He became a member in 1916, and has closely followed the vicissitudes and triumphs of the College since that time, holding several offices of trust and responsibility during the formative period of the Society, as well as in later, more sanguine years.

The History is conveniently and logically divided into two parts. Part One consists of chapters on: "The Founding of the College"; "The Presidents of the College"; "Other Officers," including The Secretary-Generals, The Treasurers, Editors of the Journal, Chairmen of the Board of Governors and The Executive Secretary from the pen of Doctor William Gerry Morgan; "The Constitution and By-Laws"; "Financial Record of the College" contributed by Edward R. Loveland; also "The Publications of the College"; "The American Board of Internal Medicine"; "My Recollections of the Period, 1925-29"; "The Story of Recent Years"; by Doctors Maurice C. Pineoffs, Walter L. Bierring, Charles F. Martin, and James Alexander Miller, respectively. This section contributes a number of short biographical sketches of outstanding American physicians. Part Two, "The Chronology," presents, in documentary form, abstracts from the minutes of the meetings of the Board of Regents and the Board of Governors. A final chapter outlines the present objectives, activities, administration, and requirements of admission to the College. The preface is splendidly written by a former President and present Secretary-General, Doctor George Morris Piersol.

A comprehensive, quarter century history of The American College of Physicians must necessarily contain a great deal of the worth-while history of American medicine. Therefore, this work should be of vital interest to all who practice that art in this country.

The Pharmacological Basis of Therapeutics*

THIS is a major contribution to the literature of pharmacology. It will undoubtedly soon assume a position as one of the three leading textbooks on the subject. The sequence of presentation is new. The drugs discussed are brought up-to-the-minute and include the latest sulfonamide compounds, endocrine preparations, and the newest anesthetics and hypnotics. The discussion of pharmacologic action is broadened in that there is more than the usual description of clinical action in addition to effects on experimental animals. The volume will find a place not only as a textbook but also as a general reference manual.

*The Pharmacological Basis of Therapeutics. A Textbook of Pharmacology, Toxicology and Therapeutics for Physicians and Medical Students. By Louis Goodman, M.A., M.D., Assistant Professor of Pharmacology and Toxicology, Yale University, School of Medicine; and Alfred Gilman, Ph.D., Assistant Professor of Pharmacology and Toxicology, Yale University, School of Medicine. Cloth, pages 1383, \$12.50. The Macmillan Company, New York, 1941.

CORRESPONDENCE

The Editor,

JOURNAL OF LABORATORY AND CLINICAL MEDICINE.

Owing to the fact that the male partner in marital infertility is of late being more acutely observed, reports emanating from competent observers show that the husband is at least responsible for sterility just as frequently as is his mate. With this new swing toward placing the responsibility upon the male in certain instances of necrozoospermia, a word of caution should be interposed.

If repeated semen analyses, when correctly performed, show no evidence whatsoever of the presence of spermatozoa, the basic cause for infertility may be placed directly upon the male. However, within the past few years I have seen the male blamed in numerous instances because of an erroneous diagnosis of total necrozoospermia. Although an ejaculate is sometimes encountered with all the spermatozoa motionless (dead), true necrozoospermia within the male genitals is a rarity. Sperms can be easily killed by a number of external factors soon after ejaculation, giving the false impression that they were dead before ejaculation. I believe that this occurs more frequently than is usually believed.

I have collected 17 illustrative patients (four of them physicians), in whom the seminal fluid had been previously tested by different examiners. In each instance, the husband had been informed that many sperms were found but that all were dead. Since these men had been found deficient, their spouses were not examined. Following the semen findings, the men were encouraged either to submit to some form of injection therapy to "improve" their sperms or to adopt a child or to resort to artificial donor inseminations.

In my examinations the semen in *every case* presented living, motile spermatozoa. Although six men showed evidence of spermiatic deficiency, not one of them had specimens that contained less than 16,000,000 living spermatozoa per cubic centimeter of semen. Eleven were found to fit within the normal range of male fertility, and in these it was urged that the etiology of the barren marriage be sought for in the female partners. Upon checking back at the earlier diagnosis of total necrozoospermia, it was shown that in each instance there was some error either in the collection or in the transportation of the semen, prior to examination. The death of the spermatozoa could, therefore, be definitely traced to some external factor following ejaculation.

By far the most common cause of death of spermatozoa after ejaculation was the condom method of semen collection. It has been repeatedly shown within the past few years that the condom should be discarded as a method of collection of semen for analytical purposes, because the chemical substances with which the rubber is treated are drastic spermicides. Not only do the substances

used in the manufacture of rubber act as spermicides, but also in many instances condoms are treated with chemical dusting powders, possible contraceptive substances, and other materials deleterious to sperm life. The use of the washed condom should also be discontinued since it is not ideal for sperm life and rubber decomposes when lubricated.

Another means used to cause neerozoospermia was the condom specimen placed in hot water. The patient in his zeal to follow instructions to "keep the specimen warm" placed the condom in a thermos bottle containing hot water which could almost scald. This, in spite of the now accepted fact that spermatozoa fare poorly in heat and do well at temperatures *below* that of the body. Spermatozoa live more than twice as long in a test tube at ordinary room temperature than when kept warm at body temperature.

Other causes of sperm death were contaminants from glass receptacles. In one instance, the patient was given a container supplied by the city health department for use in collecting sputum for acid fast studies. The patient ejaculated into this container and noted a strong odor of carbolic acid from the specimen jar. A few drops of phenol are added to these sputum jars as a preservative, and carbolic acid, even when dilute, is a strong spermicide. In another instance, I received a specimen in a container which smelled strongly of formalin. Of course, the sperm cells were motionless. In an attempt to be ultra-scientific one physician brought his own semen to be analyzed and added what he thought to be physiologic saline to the semen. All the spermatozoa were immotile. It was found that the supposed "physiologic saline" was not really normal saline but a rough approximation. Unless the concentration of saline is exactly that of normal body solution of salts, any slight variation will be hostile to sperm life. Furthermore, normal saline has no place in semen collection or analysis.

Ordinary tap water has recently been shown to be highly spermicidal. Spermatozoa die immediately when exposed to plain water. All receptacles should be absolutely dry when used for semen collection.

I suggest a simple method for collection of semen. Although it may not be the most aesthetic, it is the most exact and scientific method. After the male has refrained from intercourse for four or five days, he appears at the examiner's office. He carefully washes his hands and genitals with soap and water. Then the hands and penis are thoroughly rinsed a number of times with tap water to remove all soap. The hands and genitals are dried with a clean towel. He urinates, to free the urethra from possible soapy contaminants. A specimen is then obtained by ejaculation into a clean, sterile, dry, wide-mouthed glass receptacle (either a wide specimen jar or an ordinary glass tumbler). None of the ejaculate is lost. The semen remains in the glass receptacle at room temperature for ten to fifteen minutes, to liquefy and become mucolyzed. It is then ready for examination.

ABNER I. WEISMAN.

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PROGRESS

RENAL ASPECTS OF EXPERIMENTAL AND CLINICAL HYPERTENSION*

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ESSENTIAL and malignant hypertension were separated from the renal hypertension of Bright's disease by the accurate clinical and pathologic observations of Allbutt, Janeway, Volhard, Keith, and others. The intuitive concept of a renal origin of all, or at least of most, syndromes of increased arterial pressure was discarded because of (a) the manifest differences in clinical course which separate Bright's disease from essential and malignant hypertension; (b) the characteristic differences of degree and kind of renal structural change in nephritis and hypertension; (c) the view that, in early hypertension, the kidney is structurally and functionally intact and that, therefore, it cannot be the origin of the disease.

While the first and second of these criteria are undoubtedly true, they do not in themselves establish the extrarenal origin of essential and malignant hypertension. The third criterion, which supposes the integrity of the kidney in hypertension, seems to be invalidated by recent observations on the structure and function of the kidney in hypertension. Since the supposition of an extrarenal pathogenesis of essential and malignant hypertension largely depends on this assumption, a renal origin of hypertension is, therefore, not excluded.

Goldblatt, Lynch, Hanzal, and Summerville¹ have established that a syndrome similar in many ways to essential and malignant hypertension in man may be produced experimentally in animals as the result of partial compression of one or both main renal arteries. Experimental hypertension of apparently identical character has also been obtained by compression of the renal parenchyma in the fibrocollagenous scar of perinephritis due to application of silk or cellophane.² This development of experimental counterparts of hypertension

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has prompted an upsurge of interest in the relation of the kidney to increased arterial pressure, which has led to new concepts of the pathogenesis and clinical physiology of both the experimental and clinical disease. These concepts and methods in which they find their application form the subject of this review.

ORIGIN OF EXPERIMENTAL HYPERTENSION

Hypertension produced by compression of the renal arteries or of the renal parenchyma has been widely regarded as the result of renal ischemia. In particular, it is attributed to ischemia of functional renal tissue, since widely devitalized kidneys do not function as sites of origin of experimental hypertension.³ Measurements of renal blood flow in uninephrectomized dogs made hypertensive by compression of the renal artery have shown a decrease which averaged 40 per cent and ranged from 18 to 63 per cent.⁴ Further, it has been widely observed that experimental hypertension is more severe and more often malignant when renal arterial occlusion is nearly complete.⁵

Some doubt was cast on the belief that renal ischemia as such is the cause of experimental hypertension by the observation that urea clearance was sometimes normal in experimentally hypertensive animals,⁶ since urea clearance in normal dogs parallels the rate of renal blood flow.⁷ The further observation that the renal clearances of phenol red, inulin, and creatinine were in some instances not altered during the induction or course of experimental hypertension added weight to this doubt.⁸ Indirect measurements of renal blood flow before and after compression of the renal artery in uninephrectomized dogs with single explanted kidneys have since shown that hypertension of moderate degree may be induced without any significant or lasting change in renal blood flow (Chart 1), while in some instances renal blood flow may be reduced without the occurrence of an increase in arterial pressure.⁹

The apparent paradox that compression of the renal artery sufficient to induce changes in the kidney which lead to hypertension need not always be associated with renal ischemia demands an explanation that depends upon a consideration of the mechanism of blood flow. Thus, it should be noted that the rate of blood flow is the product of *mean* arterial pressure and of peripheral resistance. If, with increased resistance, there is a corresponding increase of pressure, no reduction of flow need occur. In any case, a high degree of arterial occlusion is necessary in order to reduce mean arterial pressure and blood flow distal to the point of compression.¹⁰ It should also be observed that the initial effect of arterial compression is a reduction of pulse pressure distal to the point of clamping, while mean arterial pressure and, therefore, blood flow, remain unchanged. It follows from these principles that arterial compression, as by the Goldblatt clamp, will nearly always result in a decrease of pulse pressure distal to the clamp and, if the constriction is sufficiently intense, will be associated with a decrease of mean pressure and blood flow. However, if mean arterial pressure proximal to the clamp is simultaneously increased, and if the compression is not excessive, neither mean arterial pressure nor renal blood flow need be reduced by clamping.

The view that renal hypertension may occur in the absence of renal ischemia is supported by the observation that mean renal arterial pressure in some experi-

mentally hypertensive dogs is normal distal to the point of clamping.¹¹ It is at least unlikely that renal blood flow would be affected by clamping when mean arterial pressure was not. Of interest also are observations¹² which demonstrate that, following clamping of the renal artery, there is a tendency for renal blood flow, which initially had decreased, to return to, or toward, normal levels.

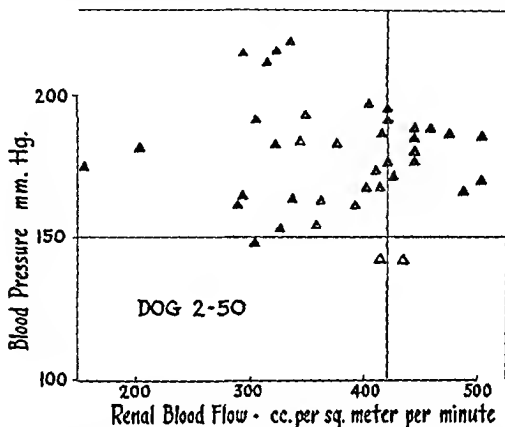


Chart 1.—Relation of mean femoral arterial pressure to total renal blood flow¹³ in an uninephrectomized dog before (Δ) and after (\blacktriangle) application of a Goldblatt clamp to the main renal artery. The wide variations in blood flow over a period of more than one year are accounted for by repeated adjustments of the degree of arterial constriction. Normal blood flow persisted during moderate hypertension.

If then, experimental renal hypertension may persist in the absence of renal ischemia, the decreased pulse pressure which follows clamping remains as a possible cause of the renal change that leads to hypertension. Experiments were, therefore, performed in which isolated dog's kidneys were perfused with blood under conditions which were as physiologic as possible. A clamp was gradually adjusted on the renal arteries to a point where pulse pressure was greatly reduced while renal blood flow was either unaffected or slightly decreased. Arterial pressure was then increased proximal to the compression so that both mean arterial pressure in the renal artery and renal blood flow remained within normal limits. Under these circumstances, the perfused kidney liberated renin, the so-called renal pressor substance, into the renal venous blood.¹³

It appears, therefore, that renal ischemia is not the necessary cause of renal hypertension, and the suggestion is made that intrarenal reduction of pulse pressure may be the exciting cause. However, it should be noted that those circumstances which excite experimental renal hypertension, possibly by reduction of pulse pressure, also, when applied with sufficient intensity, result in reduction of renal blood flow, so that the level of renal blood flow in experimental hypertension depends upon a balance struck between increased arterial pressure and increased renal arterial resistance.

The mechanism by which reduction of pulse pressure may lead to the establishment of hypertension is not clear. It is known that organs perfused in the absence of pulsatile flow speedily become edematous, and that their cells may lose their normal permeabilities to vital dyes.¹⁴ It is, therefore, conceivable that renal cells are normally impermeable to renin; this is evidenced by the absence of renin from normal renal venous blood. But reduction of pulse pressure increases renal cellular permeability so that renin escapes; this is supported by the presence of renin in the renal venous blood of hypertensive animals and man.¹⁵

If pulse pressure rather than blood flow is the determinant in the liberation of renin, it follows that renin must arise in some site normally exposed to a high pulse pressure, i.e., proximal to the glomerulus, and, probably, in an area in close relation to the afferent arteriole. Modification of the muscle cells of the afferent arterioles into larger cells which group around the glomerular vascular pole have been described (reviewed by Smith¹⁶). Although disagreement exists as to the character and normal distribution of these cells, they are regarded by some as of epithelioid nature and are stated to contain fuchsinophilic granules. Largely on the basis that these cells are hypertrophied and their granules more numerous in experimental and clinical states associated with hypertension,^{17, 18} it has been suggested that this "juxta-glomerular-myofibrillary organ" is the site of renin formation.¹⁹ Although the evidence which supports this view is entirely circumstantial, it is at least noteworthy that the position of these cells in the kidney is such that they are exposed to the extremes of pressure which occur in the afferent arterioles.

RENAL PRESSOR SYSTEM

It has been assumed in part of the above discussion that the liberation of renin somehow leads to the establishment of experimental and, possibly, of clinical hypertension. This association of renin to hypertension must now be examined.

The existence of renin, a proteinlike pressor substance in renal cortex, was first observed in 1898.²⁰ Various aspects of the original study have been confirmed and extended, largely as an aspect of the recent renewal of interest in renal hypertension. Methods of purification of renin have been described,²¹⁻²³ since crude extracts of kidney are contaminated by depressor substances.

It was observed that purer fractions of renin had little or no effect on the blood vessels of isolated organs (dog's tail, rabbit's ear) perfused with Ringer's solution,²⁴ while the addition of blood, plasma, or pseudoglobulins of plasma to renin in Ringer's solution resulted in prompt vasoconstriction. It was concluded that the pseudoglobulins of plasma contain a substance tentatively connoted renin activator. The activation of renin was then shown to be due to the interaction of renin and renin activator, with a resultant liberation of a third substance, angiotonin.²⁵ Angiotonin, unlike renin and renin activator, is not a protein. It is thermostable and dialyzable, and certain of its crystalline derivatives have been prepared. Its action as a pressor substance is both prompt and of short duration, and is, therefore, unlike the slower rise and sustained increase of arterial pressure which follow injections of renin.

Repeated injections of renin into normal animals result in a loss of pressor responsiveness (tachyphylaxis) which, in part, is due to a loss of activator by

combination with the injected renin. However, restoration of activator by transfusion or injection of activator concentrates does not fully restore the pressor action of renin. Repeated injection of angiotonin causes loss of responsiveness also, but this tachyphylaxis does not develop as rapidly as that due to injection of renin and plasma transfusion, and injection of activator does not affect it. Bilaterally nephrectomized animals are sensitized to the pressor action of renin and angiotonin and do not develop tachyphylaxis to angiotonin and only slowly to renin. Angiotonin tachyphylaxis and the residual inhibition of the pressor action of renin after restoration of activator, therefore, seemed to be due to the liberation of an angiotonin inhibitor from the kidneys.²⁸

Harrison, Grollman, and Williams,²⁷ and one of us and co-workers²⁸ have been successful in attempts to extract such an inhibitor or antipressor substance from the kidneys. The evidence at present available indicates that we are dealing with the same active principle as that reported shortly before by Harrison, Grollman, and Williams. Our work was based on different reasoning and was done independently. The two groups are now collaborating toward a further solution of the problem. The *in vitro* activity of such extracts on angiotonin (hypertensine) has recently been reported.²⁹

To summarize, the pressor system may be considered as originating in renin which, when released into the blood, combines with renin activator to yield angiotonin. Angiotonin acts as the effective vasoconstrictor and pressor agent. Opposing or balancing the action of angiotonin, the kidney contains a substance which acts as an inhibitor of the action of angiotonin. The relation of this pressor system to experimental and clinical hypertension is suggested by the following observations: (a) renin is present in the renal venous blood of hypertensive but not of normal animals; (b) the blood of hypertensive animals and human beings has properties which suggest that it contains less angiotonin inhibitor than normal blood; (c) nephrectomized and, possibly, hypertensive animals are more sensitive to the pressor action of angiotonin than are normal animals; (d) the peripheral blood of hypertensive animals and human beings contains a vasoconstrictor which, like angiotonin, is potentiated in the presence of blood from nephrectomized animals³⁰; (e) as will be discussed below, the nature of the pressor action of angiotonin is not inconsistent with that of a substance which might participate in the genesis of hypertension.

Independent and almost simultaneous confirmation of this concept of the humoral pressor system in hypertension is given by the findings of Braun-Menendez, Fasciolo, Leloir, Muñoz, Taquini, and Houssay, of Buenos Aires.³¹ These workers have also shown that a pressor substance, apparently renin, is liberated from ischemic kidneys and, proceeding from the observation that renin is not in itself vasoactive,^{24a} have shown that it interacts with a pseudoglobulin, renin activator (called by them hypertensinogen), with liberation of an effector substance, similar in physiologic properties to angiotonin, to which they refer as hypertensine. The chemical similarity or identity of angiotonin and hypertensine remains to be established. Indirect evidence has led them to the conclusion that there exists in normal kidneys an angiotonin inhibitor, called by them hypertensinase, since it is their view that it may act by enzymatic destruction of hypertensine (angiotonin).

Other pressor substances have been described as originating in the kidney. Thus, "perfusin" which, unlike renin and angiotonin, is potentiated by cocaine, has been obtained by perfusion of the kidneys of normal hogs and dogs with Ringer's solution.³² A substance, whose properties are not unlike those of renin, is found in the renal venous blood of *completely* ischemic kidneys and has been provisionally called ischemin.^{33a} It now appears that this substance is renin.^{33b} Also interesting in this connection is the observation that isolated ischemic kidneys or anaerobic kidney tissue *in vitro*,^{34a} as well as completely and partially ischemic kidneys *in vivo*,^{34b} form a pressor substance, apparently hydroxytyramine, from "dopa" (dihydroxyphenylalanine). This substance, like "perfusin," is potentiated by cocaine and may be similar to the pressor substance formed during the anaerobic incubation of kidney tissue.³⁵ These observations are of particular interest in view of the reported activity of tyrosinase in restoring to normal increased arterial pressure in hypertensive rats and dogs.³⁶ It should, however, be observed that formation of apparent hydroxytyramine is largely restricted to conditions of anaerobiosis and extreme oxygen want, whereas the oxygen content of renal venous blood in hypertensive dogs, even in the presence of moderate renal ischemia, is within normal limits.^{4, 11} It is doubtful whether anaerobic conditions could prevail in kidneys while the oxygen content of the blood remains unchanged.

RENAL FUNCTION IN HYPERTENSION

The supposed integrity of the kidney in hypertension was based largely on the absence of proteinuria and hyposthenuria and on the normal urea clearance of many hypertensives, since it was assumed that an abnormality of renal function would be revealed in one or the other of these tests. Unfortunately, hyposthenuria, when present in mild degree, may not manifest itself unless efforts are made to obtain urine of maximum specific gravity, as in the Addis concentration test, and, when such efforts are made, it can be shown that the majority of hypertensives whose urea clearance is normal show definite impairment of the maximum ability to concentrate urine (Chart 2). More recently developed methods of study of renal function have shown that even in early cases of hypertension there is evidence of some degree of renal ischemia, apparently due to constriction of the glomerular efferent arterioles. These methods, the determination of the clearances of diodrast, phenol red, and inulin, and of the maximum ability of the kidneys to secrete diodrast and reabsorb glucose, have been developed and widely applied by Smith, Shannon, Goldring, Chasis, Ranges, and their co-workers.^{16, 37}

Briefly, inulin is excreted only by glomerular filtration. Its clearance (volume of plasma equivalent to the amount of inulin which appears in the urine each minute) is, therefore, equal to the volume of glomerular filtrate formed each minute. Diodrast, on the other hand, is excreted largely by tubular secretion, i.e., by transfer of diodrast from the plasma of the peritubular capillaries across the cells of the proximal convoluted tubules into the tubule lumen. This removal of diodrast from blood in the kidney is nearly complete at low plasma concentrations of diodrast, i.e., when diodrast is excreted at less than a maximum rate. If renal diodrast load is large, so that more is present in the kidney than

can be transferred by its cells, the removal of diodrast from blood then becomes less complete, since excretion is already at a maximum. However, since at low diodrast loads extraction approaches completion (80 to 90 per cent), it follows that diodrast clearance at such loads is nearly equivalent to renal plasma flow, or, at least, to the rate of flow of plasma to functioning tubular areas (effective renal plasma flow). It may be assumed that the proportion of diodrast left in the renal blood at low renal loads (10 to 15 per cent of that present in arterial

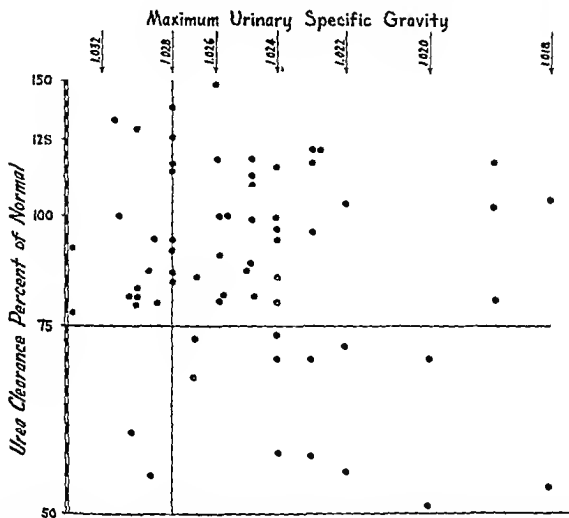


Chart 2.—Relation of urea clearance and maximum urinary specific gravity in 72 cases of essential hypertension. Abscissa (urinary specific gravity by Addis test) and ordinate (urea clearance) are plotted logarithmically. Maximum urinary specific gravity is within normal limits in only 22 instances, while normal values of urea clearance were observed in 50 patients.

blood) represents that volume of total renal blood flow which traverses supporting and nonexcreting renal tissues. A small proportion of the urinary diodrast is obtained from the red blood cells, so that it is inaccurate to assume that urinary diodrast is wholly derived from plasma. For practical purposes, since the proportion of diodrast added from the red blood cells is equivalent only to from 10 to 15 per cent of the diodrast excreted, it may be assumed that apparent plasma diodrast clearance is nearly equivalent to total renal plasma flow.^{38, 39} It follows that renal blood flow may be calculated from plasma diodrast clearance if the fraction of plasma present in the blood (1-hematocrit index) is known. Normal levels of diodrast clearance, renal blood flow, inulin clearance, and ability to excrete diodrast in man have been established.⁴⁰

Phenol red is excreted by a mechanism similar to that of diodrast, but, apparently, less efficient, since only about 50 per cent of the phenol red is removed from the blood.⁴¹ Phenol red clearance is, therefore, equivalent to roughly 50

per cent of renal plasma flow, and, in man, to about 56 per cent of plasma diodrast clearance, viz., roughly 375 c.c. per minute per 1.73 square meters of body surface.

Simultaneous measurement of inulin and diodrast clearances yield values equivalent, respectively, to the rate of glomerular filtration, i.e., the volume of water removed from the plasma by filtration; and to the volume of plasma from which the filtrate was formed. Increased intraglomerular pressure increases the proportion of water squeezed from the plasma through the glomerular capillaries, and thus increases the ratio of $\frac{\text{inulin}}{\text{diodrast}}$ clearance (filtration fraction). This ratio, which represents the fraction of plasma water filtered, is, therefore, a measure of changes of intraglomerular pressure. In hypertension, diodrast clearance, i.e., renal plasma flow, is usually decreased below normal levels, while inulin clearance is maintained. The normal level of inulin clearance depends upon increased intraglomerular pressure. The increased intraglomerular pressure of hypertension is due neither to the high level of systemic arterial pressure nor to their dilatation of the afferent arteriole, since either of these changes would of themselves increase renal blood flow, which, in hypertension, is decreased. It follows that both the decreased renal plasma flow and the maintenance of filtration rate in hypertension are the result of constriction, predominantly of the glomerular efferent arterioles.⁴²

The increased filtration fraction of hypertensives maintains glomerular filtration within normal limits long after there has occurred a marked decrease in renal blood flow, and thus explains the maintenance of normal urea clearance until late in hypertension, since urea is also excreted by glomerular filtration. This fact also may explain the observation that concentrating power usually fails before urea clearance decreases, since the concentration of urine depends upon the capacity of the renal cells to work, and it may well be that the ischemic cells of hypertensives are unable to work efficiently. The maintenance of glomerular filtration also implies that recourse to measurement of the blood levels or clearances of the other substances excreted by filtration (nonprotein nitrogen : urea nitrogen ratio, suerose, creatinine, xylose clearances) will be of no more value than determination of urea clearance. The normal urea clearance in hypertension is not an expression of functional renal integrity, since it is usually associated with renal ischemia, but, on the contrary, is usually evidence of renal vasoconstriction, which may be intense. For example, an increase of filtration fraction from 0.20, a normal level, to 0.30, a value not infrequent in essential hypertension, is enough to maintain urea clearance at 100 per cent of normal at a time when renal blood flow has fallen from a normal level of 1,000 to an ischemic level of 600 c.c. per minute.

Technically, the measurement of the simultaneous clearances of diodrast and inulin is not difficult, although it is necessarily more painstaking than routine determination of urea clearances. It is our view that the advantages of these determinations outweigh the difficulties of technique. Briefly, diodrast and inulin in sterile 0.9 per cent sodium chloride solution are given by slow intravenous infusion after the more rapid injection of a concentrated priming dose of the mixture. When it is believed that the plasma levels of diodrast and

inulin have reached a satisfactory level, which will be maintained by the infusion, the patient is catheterized, the bladder is rinsed out with sterile saline, and the urine and washings are rejected. A sample of blood is then obtained, and the bladder is again washed and urine is collected in about twenty minutes. Urine and washings are combined, and the time washing is completed is noted as the end of the first clearance period. Subsequent periods are collected in the same manner, although, with a constant infusion, sampling of blood is necessary only in every other period. We have adopted the routine collection of three urine specimens and two blood samples, and find this satisfactory unless unusual difficulty is noted in the collection of urine, in which case further collections are made. The necessity of intravenous infusion may be avoided by subcutaneous injection of a concentrated solution of diodrast, inulin, and novocain,⁴³ and the subsequent determination of clearance is made during a fall in plasma concentrations of diodrast and inulin, so that blood samples should be obtained in each period. Clearance is calculated as UV/P , where U equals urine concentration in milligrams per 100 c.c. and V is the volume of mixed urine and washings in cubic centimeters per minute, while P is the mean plasma concentration during the clearance period expressed in milligrams per 100 c.c. The value of P is obtained by interpolation from observed values to a point in each period approximately one minute before the midpoint of each period, thus allowing a delay time of one minute for the passage of urine down the renal tubules and ureters. Methods of diodrast analysis have been described by Smith, Goldring, and Chasis,⁴⁴ and by White and Rolf.⁴⁵ Methods of inulin analysis in present use are those of Alving, Rubin, and Miller,⁴⁶ and of Corcoran and Page.⁴⁷ We are at present using a slight modification of the method of White and Rolf for diodrast analysis.

The simultaneous measurement of diodrast and inulin clearance may be extended to the measurement of the maximum capacity of the kidney to secrete diodrast. Whereas efforts are made in the determination of renal plasma flow to maintain renal diodrast load (renal plasma flow \times plasma diodrast concentration) at levels such that the capacity of the kidney to transfer diodrast is not taxed and the removal of diodrast from blood is thus kept at a maximum, the determination of maximum capacity to secrete diodrast demands that the diodrast load be sufficient, so that secretory capacity of the kidney is overtaxed. Under such conditions, the diodrast excreted by the kidney by secretion (diodrast present in urine—diodrast excreted by filtration) reaches a constant level, which is expressed in milligrams per minute. The value expresses the maximum capacity of the diodrast transfer mechanism and, since the mechanism of transfer depends upon activity of the tubule cells, it follows that the maximum diodrast secretion rate is an expression of the mass of functioning tubular tissue. This value has been denominated Tm_D (tubular mass, diodrast). Clearly, interpretation of the absolute levels of diodrast and inulin clearances with reference to Tm_D is a more accurate expression of the status of renal hemodynamics than is their reference to average normal values, since thus stated, the value $\frac{\text{diodrast clearance}}{Tm_D}$ is an expression of cubic centimeters of plasma flow per unit of tubular tissue. Errors of interpretation due to differences in kidney size

in people of similar surface area are, therefore, excluded. Hypertensives, whose diodrast clearance may be normal with reference to surface area, may by this means be shown to have a decreased blood flow in cubic centimeters per unit of tubular tissue,⁴² i.e., with reference to renal mass.

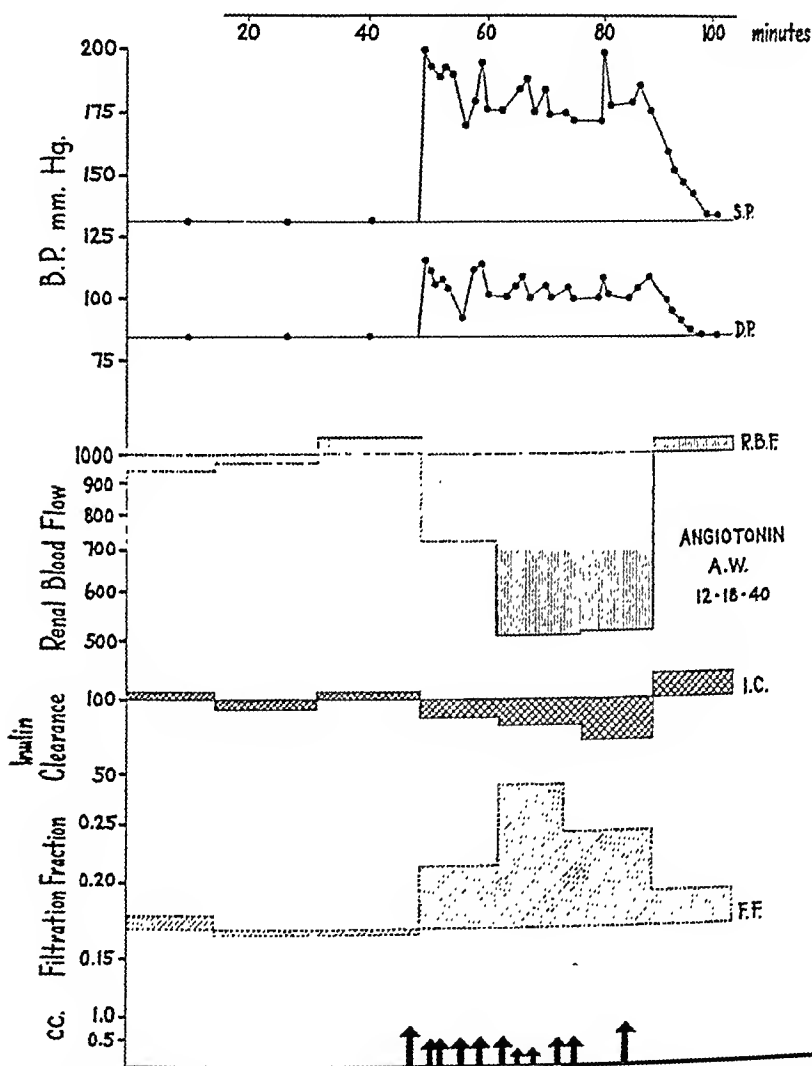


Chart 3.—Effects of infusion of angiotonin on arterial blood pressure, renal blood flow, inulin clearance, and filtration fraction in patient A. W. Ordinates from above downward: arterial systolic (S.P.) and diastolic (D.P.) blood pressure in mm. Hg.; renal blood flow (R.B.F.) in cubic centimeters per minute; inulin clearance (I.C.) in cubic centimeters per minute; filtration fraction (inulin/diodrast clearance ratio, F.F.); cubic centimeters of initial fluid. Effective intravenous injection of angiotonin and of subsequent renal blood flow, inulin clearance, and filtration fraction are plotted semilogarithmically. Abscissa: time in minutes.

Application of these methods to the study of renal function is shown in Charts 3 and 4. Chart 3, which illustrates the effect of angiotonin on renal circulation, also demonstrates the similarity of the functional change due to angiotonin to that which is present in hypertension. Injection of angiotonin in human beings increases arterial pressure and greatly decreases renal blood flow, but decreases only slightly the rate of glomerular filtration, because, as in hyper-

tension, there occurs increased extraction of water from the glomerular plasma (increased filtration fraction).⁴⁸ Angiotonin, therefore, constricts the glomerular arterioles. Its renal action is such that it may be the pressor substance of hypertension. In this connection, it should be noted that the renal ischemia

Malignant Hypertension: Effect of Renal Extracts

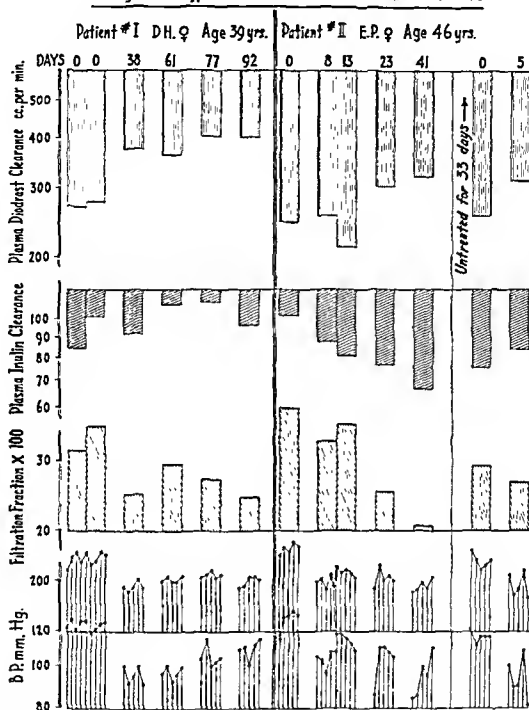


Chart 4.—Action of renal extracts on renal hemodynamics and arterial pressure in malignant hypertension. Numerals on abscissa refer to days in each series of intramuscular injections of renal extracts, while ordinates are referred to average normal values.

of hypertension, if due to angiotonin, is more probably a result of the action of the vasopressor system than a cause of the condition. A similar, apparently secondary, renal hemodynamic abnormality is present in the diastolic hypertension associated with coarctation of the aorta.⁴⁹ A further similarity of angiotonin to the possible pressor agent of hypertension, and one which excludes adrenin and pituitrin, is the fact that angiotonin exerts its pressor action without causing pallor or a decrease of skin temperature.⁵⁰ This is also true of renin.^{41, 51} Others have shown that the acute pressor action of angiotonin is associated with greatly increased peripheral resistance and with decreased

cardiac output⁵² and increased venous pressure,⁵³ the latter possibly the result of the heart's emptying against great resistance. Angiotonin also increases the force of the heartbeat⁵⁴ and cardiac work and efficiency, but it decreases diastolic cardiac volume.⁵⁵ It is interesting to speculate as to whether or not angiotonin would decrease cardiac output in the case of the hypertrophied heart of a long-standing hypertensive person.

Studies of normal renal hemodynamics by the clearances of diodrast and inulin have shown an apparent autonomy of renal circulation, which is largely exercised by variations in caliber of the efferent arterioles.⁵⁶ The normal tendency of the autonomous renal circulation is to respond to variations of systemic blood pressure and supply by changes of efferent arteriolar tone which maintain filtration rate near its normal level, at the same time varying the rate of renal blood flow. Since, as has been noted, renin may be liberated in response to pressure changes in the afferent arteriole, it is not illogical to assume that the renal vasopressor system may have a normal function in the autonomy of renal circulation, and hypertension may be an abnormal release of this mechanism. Teleologically, it is difficult to imagine a function of this system in the maintenance of normal arterial pressure, since the normal blood pressure level is not affected by bilateral nephrectomy.

Diodrast and inulin clearances have also been used in the study of the renal changes that result from the treatment of patients suffering from hypertension with renal extracts containing the renal antipressor, or with other agents. The decreased arterial pressure following sympathectomy in hypertension,^{57, 58} or the therapeutic use of thiocyanate,⁵⁹ is not usually associated with the undoing of the efferent arteriolar constriction, and it may be assumed from this that neither sympathectomy nor thiocyanate reverses the process that leads to increased arterial pressure and renal vasoconstriction.

The action of renal antipressor extracts, on the contrary, is associated with a concurrent decrease of arterial pressure and evidence of efferent arteriolar relaxation, an observation that adds to the evidence which suggests that these extracts in fact inhibit the action of angiotonin and the renal vasopressor system in hypertension⁶⁰ (Chart 4). The extent to which renal blood flow is affected by renal vasodilatation depends upon the balance between renal vasoconstriction, vasosclerosis, and arterial pressure, so that it is not greatly increased when renal vasodilatation is associated with decreased arterial pressure.

The measurement of diodrast, phenol red, and inulin clearances has also yielded interesting information as to the renal functional changes present in pre-eclamptic and eclamptic toxemia of pregnancy.⁶¹ The succession of these changes in two patients with eclampsia before and after delivery is shown in Chart 5. Briefly, pre-eclampsia and eclampsia were characterized by a decrease of filtration fraction, which seems to be an expression of swelling of the glomerular capillaries, since it is not usually associated with decreased renal blood flow. Other patients suffering from "toxemia of pregnancy" show an increased filtration fraction and a decreased renal blood flow. It is believed that in these latter the renal changes are an expression of a process identical with that of essential hypertension.

It will be noted that the afferent arteriole has been largely neglected in the foregoing discussion, in which emphasis is placed on constriction of efferent arteriole in hypertension. Structural, as opposed to functional, studies of the kidney in early hypertension have shown that the characteristic lesion is not situated in the efferent arteriole, but that it consists of hyalinization and sclerosis

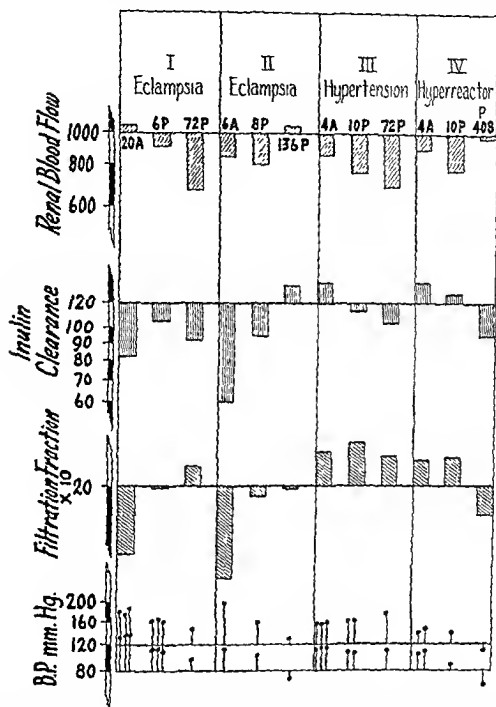


Chart 5.—Renal hemodynamics and arterial pressure in "toxemia of pregnancy." The numerals on abscissa refer to the day of observation in number of days ante (A) or postpartum (P). These cases were referred to elsewhere⁶¹ as patients No. A1, A2, B4, and B3.

of the afferent arteriole. Afferent arteriolar sclerosis may, in fact, be widespread at a time when there is little evidence of abnormal arteriolosclerosis elsewhere in the body.⁶² A possible explanation of this curious opposition of structural and functional changes may be obtained from consideration of vasosclerosis as it occurs in experimental renal hypertension. In this condition, the kidney is exposed, as are other organs, to the vasoconstrictor, apparently angiotonin or a similar substance, which is present in systemic blood, while it is at the same time protected from the increase of arterial pressure by the restriction of a clamp on the renal artery or a scar around the parenchyma. The kidneys of such animals rarely show evidences of sclerosis or of arteriolonecrosis in malignant hypertension, although these changes are widespread in other organs⁵ and

may be present in the contralateral "normal kidney."⁶³ The vascular degenerative changes are, therefore, apparently due in part to the increase of systemic arterial pressure and not alone to the presence of vasoconstriction. It is, therefore, altogether fitting that sclerosis should occur in the afferent (high pressure) arteriole at a time when it is absent in the efferent (low pressure) arteriole of the kidney.

The early afferent arteriolar sclerosis of hypertension has at the same time a significance other than that of a simple result of the action of increased arterial pressure, for, if it were present from other causes *before* the onset of hypertension, it would act effectively as would "myriads of little clamps."⁶⁵ Evidence of such a change prior to the onset of hypertension is unavailable, although the wide distribution of renal sclerosis in early hypertension is at least suggestive.⁶²

The final origin of hypertension remains obscure, for the demonstration of the part played by the renal pressor system clarifies some aspects of its etiology but, at the same time, poses new problems.

REFERENCES

1. Goldblatt, H., Lynch, J., Hanzal, R. F., and Summerville, W. W.: The Production of Persistent Hypertension in Dogs, *Am. J. Path.* 9: 942, 1933.
2. Page, I. H.: The Production of Persistent Arterial Hypertension by Cellophane Perinephritis, *J. A. M. A.* 113: 2046, 1939.
3. Friedman, M., and Katz, L. N.: Renal Insufficiency Following Trypsin Injections Into Renal Arteries, *J. Exper. Med.* 69: 485, 1938.
4. Levy, S. E., Light, R. A., and Blalock, A.: The Blood Flow and Oxygen Consumption of the Kidney in Experimental Renal Hypertension, *Am. J. Physiol.* 122: 38, 1938.
5. Goldblatt, H.: Experimental Hypertension Induced by Renal Ischemia, *The Harvey Lectures*, Baltimore, 1938, Vol. 33, page 237.
6. Goldblatt, H., Lynch, J., Hanzal, R. F., and Summerville, W. W.: Studies on Experimental Hypertension. I. The Production of Persistent Elevation of Systolic Blood Pressure by Means of Renal Ischemia, *J. Exper. Med.* 59: 347, 1934.
7. Van Slyke, D. D., Rhoads, C. P., Hiller, A. M., and Alving, A. S.: Relationships Between Urea Excretion, Renal Blood Flow, Renal Oxygen Consumption and Diuresis; the Mechanism of Urea Excretion, *Am. J. Physiol.* 109: 324, 1934.
8. Coreoran, A. C., and Page, I. H.: Observations on the Relation of Experimental Hypertension to Renal Clearance and Renal Ischemia, *Am. J. Physiol.* 123: 1938.
9. Coreoran, A. C., and Page, I. H.: Renal Blood Flow in Experimental Hypertension Due to Constriction of the Renal Artery, *Proc. Am. Physiol. Soc.* 53: 61, 1941.
10. Mann, F. C., Herrick, J. F., Essex, H. E., and Baldes, E. J.: The Effect on Blood Flow of Decreasing the Lumen of a Blood Vessel, *Surgery* 4: 249, 1938.
11. Mason, M. F., Robinson, C. J., and Blalock, A.: Studies on the Renal Arterial Blood Pressure and the Metabolism of Kidney Tissue in Experimental Hypertension, *J. Exper. Med.* 72: 289, 1940.
12. a. Levy, S. E., Robinson, C. J., and Blalock, A.: The Effect of Altering the Renal Blood Pressure and Blood Flow on the Glomerular Filtration of a Transplanted Kidney in Unanesthetized Dogs, *Am. J. Physiol.* 123: 383, 1938.
b. Schroeder, H. A., and Steele, J. M.: Behavior of Renal Blood Flow After Partial Constriction of the Renal Artery, *J. Exper. Med.* 72: 707, 1940.
13. Kohlstaedt, K. G., and Page, I. H.: The Liberation of Renin by Perfusion of Kidneys Following Reduction of Pulse Pressure, *J. Exper. Med.* 72: 201, 1940.
14. McMaster, P. D., and Parsons, R. F.: The Effect of the Pulse Upon the Formation and Flow of Lymph, *J. Exper. Med.* 68: 353, 1938.
15. Page, I. H.: Demonstration of the Liberation of Renin Into the Blood Stream From Kidneys of Animals Made Hypertensive by Cellophane Perinephritis, *Am. J. Physiol.* 130: 22, 1940.
16. Smith, H. W.: Physiology of the Renal Circulation, *The Harvey Lectures*, Baltimore, 1939-40, Vol. 35, page 166, Williams & Wilkins Co.
17. Goormatigh, N.: Histological Changes in the Ischemic Kidney. With Special Reference to the Juxtaglomerular Apparatus, *Am. J. Path.* 16: 409, 1940.
18. Graef, I.: Quoted by Smith.¹⁶
19. Goormatigh, N., and Grimson, K. S.: Vascular Changes in Renal Ischemia. Cell Mitosis in the Media of Arteries, *Proc. Soc. Exper. Biol. & Med.* 42: 227, 1939.

20. Tigerstedt, R., and Bergman, P. G.: Niere und Kreislauf, Skandinav. Arch. f. Physiol. 8: 223, 1898.
21. Helmer, O. M., and Page, I. H.: Purification and Some Properties of Renin, J. Biol. Chem. 127: 757, 1939.
22. McEwen, E. G., Harrison, S. P., and Ivy, A. C.: Tachyphylaxis to Renin, Proc. Soc. Exper. Biol. & Med. 42: 254, 1939.
23. Swingle, W. W., Taylor, A. R., Collins, W. N., and Hays, H. W.: Preparation and Bioassay of Renin, Am. J. Physiol. 127: 768, 1939.
24. a. Kohlstaedt, K. G., Helmer, O. M., and Page, I. H.: Activation of Renin by Blood Colloids, Proc. Soc. Exper. Biol. & Med. 39: 214, 1938.
b. Page, I. H., Kohlstaedt, K. G., and Helmer, O. M.: The Activation of Renin by Blood, Am. Heart J. 19: 92, 1940.
25. Page, I. H., and Helmer, O. M.: A Crystalline Pressor Substance (Angiotonin) Resulting From the Reaction Between Renin and Renin-Activator, J. Exper. Med. 71: 29, 1940.
26. Page, I. H., and Helmer, O. M.: Angiotonin-Activator, Renin- and Angiotonin-Inhibitor, and the Mechanism of Angiotonin Tachyphylaxis in Normal Hypertensive, and Nephrectomized Animals, J. Exper. Med. 71: 405, 1940.
27. a. Grollman, A., Harrison, T. R., and Williams, J. R., Jr.: Humoral Agents in the Causation of Hypertension, in "Blood, Heart, and Circulation," Symposium, Washington, D. C., Science Press, 1940, page 274.
b. Harrison, T. R., Grollman, A., and Williams, J. R., Jr.: The Antipressor Action of Renal Extracts and Their Capacity to Reduce Blood Pressure of Hypertensive Rats, Am. J. Physiol. 128: 716, 1940.
c. Grollman, A., Harrison, T. R., and Williams, J. R., Jr.: Therapeutics of Experimental Hypertension, J. Pharmacol. & Exper. Therap. 69: 76, 1940.
d. Harrison, T. R., Grollman, A., and Williams, J. R., Jr.: Reduction of Elevated Blood Pressure by Administration of Renal Extracts, J. A. M. A. 115: 1169, 1940.
28. a. Page, I. H.: Newer Aspects of Experimental Hypertension, in "Blood, Heart, and Circulation," Symposium, Washington, D. C., Science Press, 1940, page 239.
b. Page, I. H., Helmer, O. M., Kohlstaedt, K. G., Fouts, P. J., Kempf, G. F., and Corcoran, A. C.: Substance in Kidneys and Muscles Eliciting Prolonged Reduction of Blood Pressure in Human and Experimental Hypertension, Proc. Soc. Exper. Biol. & Med. 43: 722, 1940.
c. Page, I. H., Helmer, O. M., Kohlstaedt, K. G., Fouts, P. J., and Kempf, G. F.: Reduction of Arterial Blood Pressure of Hypertensive Patients and Animals With Extracts of Kidneys, J. Exper. Med. 73: 7, 1941.
29. Fasciolo, J. C., Leloir, L. F., Munoz, J. M., and Braun-Menendez, E.: La hipertensinasa: Su dosaje y distribucion. Rev. Soc. argent. de biol. 16: 643, 1940.
30. Page, I. H.: The Vasoconstrictor Action of Plasma From Hypertensive Patients and Dogs, J. Exper. Med. 72: 301, 1940.
31. a. Braun-Menendez, E., Fasciolo, J. C., Leloir, L., and Muñoz, J. M.: La substancia hipertensora de la sangre del riñon isquemado, Rev. Soc. argent. de biol. 15: 420, 1939.
b. Braun-Menendez, E., Fasciolo, J. C., Leloir, L., and Muñoz, J. M.: The Substance Causing Renal Hypertension, J. Physiol. 98: 283, 1940.
c. Houssay, B. A.: Mecanismo de accion de la secrecion hipertensora del riñon, Medicina 1: 3, 1941.
32. Williams, J. R., Jr., and Grossman, E. B.: Recovery of an Adrenalin-like Substance From Kidney, Am. J. Physiol. 123: 364, 1939.
33. a. Prinzmetal, M., Lewis, H. A., and Leo, S. D.: Etiology of Hypertension Due to Complete Renal Ischemia, Proc. Soc. Exper. Biol. & Med. 43: 696, 1940.
b. Prinzmetal, M., Lewis, H. A., and Leo, S. D.: The Etiology of Hypertension Due to Complete Renal Ischemia, J. Exper. Med. 72: 763, 1940.
34. a. Bing, R. J.: Formation of Hydroxytyramine by Extracts of Renal Cortex and by Perfused Kidneys, Am. J. Physiol. 132: 497, 1941.
b. Bing, R. J., and Zucker, M. B.: Acute Renal Hypertension Produced by an Amino Acid, Proc. Am. Physiol. Soc. 53: 26, 1941.
35. Victor, J., Steinitz, A., and Weeks, N. M.: A Pressor Substance Produced by Anaerobic Incubation of Renal Cortex, Proc. Soc. Exper. Biol. & Med. 42: 767, 1939.
36. a. Schroeder, H. A.: Effect of Tyrosinase on Blood Pressures of Hypertensive Rats, Proc. Soc. Exper. Biol. & Med. 44: 172, 1940.
b. Schroeder, H. A., and Adams, M. H.: The Effect of Tyrosinase on Experimental Hypertension, J. Exper. Med. 73: 531, 1941.
37. a. Smith, H. A.: Physiology of the Kidney, New York, 1937, Oxford Univ. Press.
b. Smith, H. A.: Physiology of the Kidney, The Porter Lectures, Univ. of Kansas, 1939.
38. a. White, H. L., and Heinbecker, P.: Interpretation of Diodrast Clearances in the Dog, Proc. Soc. Exper. Biol. & Med. 43: 8, 1940.
b. White, H. L., Findley, T., and Edwards, J. C.: Interpretation of Diodrast Clearances in Man, Proc. Soc. Exper. Biol. & Med. 43: 11, 1940.

39. Corcoran, A. C., Smith, H. W., and Page, I. H.: Unpublished observations.
40. Goldring, W., Chasis, H., Ranges, H. A., and Smith, H. W.: Relations of Effective Renal Blood Flow and Glomerular Filtration to Tubular Excretory Mass in Normal Man, *J. Clin. Investigation* 19: 739, 1940.
41. Corcoran, A. C., and Page, I. H.: The Effects of Renin, Pitressin, and Pitressin and Atropine on Renal Blood Flow and Clearance, *Am. J. Physiol.* 126: 354, 1939.
42. Goldring, W., Ranges, H. A., Chasis, H., and Smith, H. W.: Effective Renal Blood Flow and Functional Excretory Tubular Mass in Essential Hypertension, *J. Clin. Investigation* 17: 505, 1938.
43. Findley, T., and White, H. L.: Measurement of Diodrast and Inulin Clearances in Man After Subcutaneous Administration, *Proc. Soc. Exper. Biol. & Med.* 45: 623, 1940.
44. Smith, H. W., Goldring, W., and Chasis, H.: The Measurement of the Tubular Excretory Mass, Effective Blood Flow and Filtration Rate in the Normal Human Kidney, *J. Clin. Investigation* 17: 263, 1938.
45. White, H. L., and Rolf, D.: A Micro-Method for Determining Diodrast and Inorganic Iodide in Blood and Urine, *Proc. Soc. Exper. Biol. & Med.* 43: 1, 1940.
46. Alving, A. S., Rubin, J., and Miller, B. F.: A Colorimetric Method for the Determination of Inulin in Blood and Urine, *J. Biol. Chem.* 127: 609, 1939.
47. Corcoran, A. C., and Page, I. H.: Applications of Diphenylamine in the Determination of Levulose in Biological Media, *J. Biol. Chem.* 127: 601, 1939.
48. Corcoran, A. C., Kohlstaedt, K. G., and Page, I. H.: Changes of Arterial Blood Pressure and Renal Hemodynamics by Injection of Angiotonin in Human Beings, *Proc. Soc. Exper. Biol. & Med.* 46: 244, 1941.
49. Friedman, M., Selzer, A., and Rosenblum, H.: The Renal Blood Flow in Coarctation of the Aorta, *J. Clin. Investigation* 20: 107, 1941.
50. Corcoran, A. C., and Page, I. H.: The Effects of Angiotonin on Renal Blood Flow and Glomerular Filtration, *Am. J. Physiol.* 130: 335, 1940.
51. Landis, E. M., Montgomery, H., and Sparkman, D.: Effects of Pressor Drugs and of Saline Kidney Extracts on Blood Pressure and Skin Temperature, *J. Clin. Investigation* 17: 189, 1938.
52. Bradley, S. E., and Parker, B. A.: Effect of Angiotonin on Circulatory Dynamics, *Proc. Am. Physiol. Soc.* 53: 33, 1941.
53. Wilkins, R. W., and Duncan, C. N.: The Nature of the Arterial Hypertension Produced in Normal Subjects by the Administration of Angiotonin, *J. Clin. Investigation* 20: 442, 1941.
54. Andrus, E. C., and Hill, W. H. P.: Effect of Renin and Angiotonin Upon Isolated Perfused Heart, *Proc. Soc. Exper. Biol. & Med.* 44: 213, 1940.
55. Lorber, V., and Visscher, M. B.: The Action of Angiotonin on the Completely Isolated Mammalian Heart, *Proc. Am. Physiol. Soc.* 53: 177, 1941.
56. Smith, H. W., Rovenstine, E. A., Goldring, W., Chasis, H., and Ranges, H. A.: The Effects of Spinal Anesthesia on the Circulation in Normal Unoperated Man With Reference to the Autonomy of the Arterioles and Especially Those of the Renal Circulation, *J. Clin. Investigation* 18: 319, 1939.
57. Adams, W., Alving, A. S., Sandiford, I., Grimson, K. S., and Scott, C.: The Effect of Bilateral Paravertebral Sympathectomy on the Cardiorenal System in Essential Hypertension, *Proc. Am. Physiol. Soc.* 53: 2, 1941.
58. Corcoran, A. C., and Page, I. H.: Renal Blood Flow and Sympathectomy in Patients Suffering From Hypertension, In press.
59. Corcoran, A. C., and Page, I. H.: Unpublished Observations.
60. Corcoran, A. C., and Page, I. H.: Effects of Renal Extracts Containing "Angiotonin-Inhibitor" on Renal Blood Flow and Function in Normal and Hypertensive Dogs and Human Beings, *Proc. Am. Physiol. Soc.* 53: 60, 1941.
61. Corcoran, A. C., and Page, I. H.: Renal Function in Late Toxemia of Pregnancy, *Am. J. M. Sc.* 201: 385, 1941.
62. Moritz, A. R., and Oldt, M. R.: Arteriolar Sclerosis in Hypertensive and Nonhypertensive Individuals, *Am. J. Path.* 13: 679, 1937.
63. Wilson, C., and Byrom, F. B.: Renal Changes in Malignant Hypertension, *Lancet* 1: 136, 1939.

Addendum: Recently published observations correlate the diastolic arterial pressure of patients with hypertension with the rates of renal blood flow and glomerular filtration (Friedman, M., Selzer, A., Rosenblum, H.: The Renal Blood Flow in Hypertension, *J. A. M. A.* 117: 92, 1941). A new method for the determination of diodrast has also been described (Alpert, L. K.: A Rapid Method for the Determination of Diodrast-Iodine in Blood and Urine, *Bull. Johns Hopkins Hosp.* 68: 522, 1941).

CLINICAL AND EXPERIMENTAL

CAPILLARY STUDIES IN RAYNAUD'S DISEASE¹

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INTRODUCTION

EXACT capillaroscopic investigations and functional tests in cases of Raynaud's disease in general, as well as in those studied before and after complete elimination of sympathetic innervation, promise to yield significant data as to the role of the autonomic influences on the capillary. The relationship to the neuroses which have found their symptomatic expression in this part of the circulatory system may also be elucidated.

This study is an investigation of the constitutional capillary disturbances, the functional processes in the capillaries and the capillary permeability in cases of Raynaud's disease. These factors were studied both before and after preganglionic sympathectomy.

It is impossible to discuss the work of the many outstanding investigators who have been interested in these problems from the physiologic and psychologic viewpoints.^{1-6, 11, 13-16, 19, 22, 23, 31, 33, 36} Raynaud himself suggested that emotional stress can precipitate an angiospastic attack.²⁹ In recent years the most extensive survey of the various concepts of Raynaud phenomena has been published by Hunt¹⁷ who states that in 50 per cent of the cases of Raynaud's disease the attacks are precipitated by emotional factors (surprise, fatigue, etc.). He denies, however, any relationship between this illness and the psychoneuroses. That psychic stimuli may cause attacks is a point also stressed by Simpson, Brown, and Adson,³⁰ and by White.³³ Recently, Mittelman and Wolff²⁵ discussed the interplay between affective and physical environmental influences in a case of Raynaud's disease in which they could show the significance of emotional factors in the precipitation of the attacks.

Mueller and Parisius²⁷ were among the first to call attention to the relations of capillary disorders to the neuroses. They found "imbalance, disharmony, and dysergia in the psychic as well as in the organic sphere" to be characteristic for the vasoneuroses in general. These features are manifested in the form and function of the capillaries. According to these authors, a very frequent characteristic of the vasoneuroses is the "spastic-atonie symptom complex," "vasoneurotic diathesis," that is, not only prolonged widening and narrowing of the capillary lumina, but also, either simultaneously or in quick alternation, dilata-

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tion and constriction. In all the more severe vasoneuroses the flow is extremely changeable and irregular. At times the stream flows quietly and evenly, then very quickly; at times it is sluggish and "granular," then it ceases completely for a brief interval. Often these various phenomena can be seen simultaneously in different capillary loops in the same microscopic field. Often a continuous capillary flow becomes granular. The "granular flow," in addition, may begin in the arterial as well as the venous limb. At times even spontaneous refluxes from the venous to the arterial limb are found, a phenomenon previously thought to be seen only in marked congestive failure.

In the majority of vasoneurotics Mueller and Parisius found an increased number of somewhat congested papillary capillaries and more vessels in the subpapillary plexus. (They examined the skin of the interclavicular groove.) In a small number of cases no abnormal overfilling of the vessels was seen; on the other hand, often no capillaries at all were visible. The size of the capillaries also deviates from the norm. Frequently there are long and thin loops, at times very short, thick, irregularly distributed loops. The most striking, and indeed the most pathognomonic, capillary form which is found in vasoneurotics is the irregular rete mirabile. At the nailfold the loops are not quite parallel; rather, they converge or diverge in an indefinite fashion. As a rule, the arterial limb is very narrow, the venous rather wide and irregular; a variable number of anastomoses connect the individual loops. Finally, the subpapillary plexus is usually visible and shows very wide vessels. Besides the characteristics mentioned above, according to Mueller and Parisius, there is also a functional disturbance in the permeability of the capillary walls which can be seen microscopically in the more severe forms of the vasoneuroses, is the hemorrhagic diapedesis, occurring either spontaneously or after slight mechanical trauma. The above capillary picture, as defined by Mueller and Parisius, was later confirmed by many other authors.

MATERIAL

From the capillaroscopic investigations of a series of cases presenting pathologic conditions, 42 patients, 29 of whom had Raynaud's disease, were selected for the purposes of this study. The other 13 patients were included either for purposes of comparison because of the similarity of the symptomatic picture, as, for example, Buerger's disease, or because, for one reason or another, a sympathectomy was performed and they, therefore, seemed to be of particular interest.

Forty-two hands of nonoperated patients were examined. Forty-six hands were of patients who had had a sympathectomy at the time of examination; 16 of these had also been seen before operation. Frequently the same patient was examined several times, particularly if an operation had been performed, in order to be able to observe the capillary changes at various times postoperatively.

METHOD

Before the capillaroscopic examination was performed, the following clinical data were obtained: The body habitus and its deviation from the standard types was noted. The color, pigmentation, approximate temperature, degree of moisture or dryness, fat content and separability of the skin from the subcutaneous

tissue were indicated. The skin was tested for dermatographism. The vasomotor instability was checked by the absolute pulse rate and by the respiratory arrhythmia during inspiration and inspiratory apnea (Valsalva test). The systolic and diastolic pressures and their response to pressure on the carotid sinus were recorded.

CAPILLAROSCOPY

A Zeiss capillaroscope with a 60 \times objective and a 10 \times eyepiece was used. Since the small lamp within the tube does not offer adequate elimination, a special lighting apparatus for this purpose was attached. A special attachment permitting simultaneous observation and photography was inserted.

The morphology of the capillaries was described, with particular reference to developmental disturbance (archicapillaries, subcapillary plexus).¹⁸ The corium was described as being well scalloped, not well scalloped or straight.

The number of capillaries per field in the first row was counted.

The length and width of the capillaries and changes in their appearance were noted.

The flow in the capillaries was described as granular or uniform, slow or fast, intermittent or continuous, and the appearance of "gaps" and periods of stasis were noted.

In addition, the following tests were done in order to gain better insight into the functional responses: The flow in the capillaries was studied after occluding the arterial and venous flow by the use of a blood pressure cuff quickly pumped up to 30 mm. above systolic blood pressure. The "reflux time,"³⁴ the eventual reversal in flow from the venous side to the arterial and any other changes appearing in this experiment were noted, with particular attention to the widening, narrowing, or disappearance of capillaries.

Finally, by slowly reducing the pressure in the sphygmomanometer cuff (about 10 mm. per 2 seconds), the height of the pressure at which the capillary flow reappeared was determined. This value we termed "critical capillary pressure."

A phenomenon to which special attention was paid was the occasional appearance of a light-refracting, collagenous tissue fluid which exuded from the large capillaries and obscured them. This was seen spontaneously as well as in the tests for reflux time. As will be explained later, this may be regarded as direct expression of increased capillary permeability.

With a special photographic apparatus, a series of colored films was taken which recorded the capillaries themselves in addition to the shading of the surrounding tissue. However, more distinct pictures were obtained by placing a green filter in front of the light, and the black and white photograph with panchromatic Agfa or Kodak film combined the advantage of a clear, sharp picture, with facility in making any desired enlargement.

When it was of no particular importance to record the width of the capillaries, but of more importance to obtain the general outline, a slight congestion of the capillaries, produced by the blood pressure cuff (50 to 60 mm.) was shown to be most advantageous in obtaining clear pictures. Of course, such photographs, taken in a congested state, cannot be used for the determination of the

actual width of the capillaries; on the other hand, if anastomoses to the vessels of the subpapillary plexus are visible microscopically, these become much clearer and are then more easily photographed.

An attempt on the part of different observers to draw the picture the same size as seen in the microscope will give varied results and will lead to pictures of very different dimensions. Keeping in mind Krogh's criticism¹⁰ of the existing capillary pictures in the literature and the lack of data on the degree of magnification, we used the following method in our study: We set up a counting chamber for red blood cells under the microscope and photographed it; thus we had a sharply delineated scale for all sharply focused capillaries. Our original photographs were thus magnified 18 \times .

By means of the picture of the counting chamber it was not difficult to prepare enlargements giving a definite magnification of the actual size of the object. We merely had to place a scale on the projection surface of the enlargement apparatus and arrange it in such a way that, for example, the intermediate space of the counting chamber which is $\frac{1}{20}$ mm. long, appeared exactly 2 cm. long, in order to obtain a 400 \times enlargement.

By this method errors in the determination of the size of the microscopic picture can be avoided. Brown⁵ made such errors; he later had to correct his measurements because of incorrect calculation of the scale. Similarly, the photographs of Duryee and Wright¹¹ are incorrectly computed. In their paper two photographs are reproduced with the statement that the enlargement is "176 times on the original film" and "the enlargements shown are about four times the size of the original film." That means then that the picture reproduced was enlarged 176×4 or 704 \times . In order to check their data we measured the largest capillary loop in their picture; it was 5 cm. long. In reality it would then be $50/704$, or 0.071 mm. long. This would be only 10 \times as large as the average red blood cell. There must, therefore, be a mistake in their calculation.

REFLUX TIME AND CRITICAL CAPILLARY PRESSURE

The method used is to encircle the upper arm with a blood pressure cuff which is quickly pumped up to 30 mm. above systolic blood pressure. The time required for the blood in the capillary under observation to stop flowing is the "reflux time," which under normal conditions averages fifteen seconds. Lange¹² found a lower value in normal persons. We must go well above systolic pressure to be sure the arterial flow is completely shut off. It is important to wait several minutes before the test is repeated, because this length of time is necessary before the venous congestion completely disappears.

When the compression is released, the capillary flow reappears in normal persons at a pressure between 10 and 25 mm. below the systolic blood pressure. This we have termed the "critical capillary pressure." A reappearance above or below these limits must be considered pathologic.

NORMAL CAPILLARY PICTURES IN ADULTS

By means of capillaroscopy one sees the following picture at the nailfold of normal adults: The boundary between the cutis and corium is well scalloped. The capillaries of the first row (*limbus capillaries*) are, for the most part, hair-

pin-like; some show loops or figure-of-eight forms. They are usually parallel. The arterial limb is narrower than the venous and allows blood corpuscles to pass through only one by one. According to Mueller,²⁰ the patent width of the lumen is 0.009 to 0.012 mm. The venous limb is wider and allows two or more blood cells in a row to pass through. The width of the lumen, according to Krogh,¹⁹ is 0.02 mm.

The height of the capillaries, that is, the straight line between the foot of the capillary, where it is just visible, to the convexity of the arteriovenous transitional loop is about 0.2 to 0.4 mm. The distance of the capillaries from one another is approximately uniform. The number of capillaries in the first row is about 7 to 10 per millimeter. The distance of the heads of the capillaries from the corium is about 0.1 to 0.3 mm. The connection between these capillaries and deeper vessels is normally not visible.

Behind the above-described row of capillaries, which is just adjacent to the corium, there is usually a second, and often a third, row of shorter, but otherwise similarly formed capillaries. In the deeper layers no arrangement in rows can be made out, but one sees rather chessboard-like patterns or scattered, short loops which represent the capillary heads.

Normally the subcapillary plexus is invisible.

The above-mentioned loops and figure-of-eight forms, if present in the minority, are considered normal; so, too, are slight deviations from the very regular structure, such as slight variations in the length of the capillaries or a slightly unequal spacing.

The flow in normal capillaries is usually smooth and even and of moderate speed, but a "granular" type of stream is sometimes seen and occasionally isolated capillaries seem to disappear. Actually this is due to the fact that they contain no blood corpuscles, since it is only these that are visible and not the capillary itself. At other times the circulation in isolated capillaries ceases entirely for a brief period of time. Both of these phenomena are to be considered normal.

CAPILLARY PICTURE IN RAYNAUD'S DISEASE

The appearance and behavior of the capillaries in Raynaud's disease depend on the severity of the disease and are different in the different color stages of the extremities. In order to have comparable observations one must always perform the examinations in a warm room at a standard temperature. In this warm room the capillaries observed at the nailfold of a patient showed the same picture, even on different days, as long as no operation was performed.

The typical picture in a severe case of Raynaud's disease (in a warm room) is the following: Most of the capillaries are enlarged. The presence of giant capillaries with a width of 5 to 8 red blood cell diameters or more is striking. The space between the two limbs of such a giant capillary is about 10 red blood cell diameters. Its shape may be ringlike or hairpin-like or irregular and tortuous. The flow in the capillaries is slow and sluggish, and often prolonged periods of stasis are observed. In some cases blood extravasates and thromboses are found. Very often there are very narrow, thin capillaries between the enlarged, giant ones.

In severe cases which are combined with sclerodermic changes, all the above-mentioned findings may be present, and in addition, the number of capillaries is smaller. The picture is very often hazy and the flow is barely visible; the stream, if visible, is very sluggish. In the reflected light the tissue has a brighter color than in other cases, probably due to the increased collagenous fibers. In addition, the excretory ducts of the sweat glands are often visibly enlarged and dilated, and the escape of drops of sweat may be seen. Since we observed this phenomenon in several cases, it may be evidence that there is a hyperfunction of the sympathicus in Raynaud's disease. This confirms Raynaud's original concept²⁹ that one of the mechanisms of the disease is an abnormal vasomotor tone. Lewis' opinion²³ is that in true Raynaud's disease there is a "local fault" and that the vasomotor tone is normal. Bishop, Heinbecker, and O'Leary² believe in a local, inherent, trophic, etiologic factor. In some advanced cases a sensitization to humoral substances is present and is sufficient to produce an attack.

In early and mild cases of Raynaud's disease the picture is not so striking; giant capillaries, thromboses, and blood extravasates are absent. The typical picture in mild cases of Raynaud's disease or vasoneurosis is as follows: very tortuous, twisted and bent capillaries, usually with a spastic, narrow, arterial portion and a dilated, congested, venous portion. A congested subpapillary plexus is usually visible.

REFLUX TIME

In severe cases of Raynaud's disease the reflux time shows a marked deviation from the norm. We frequently find capillaries in which, upon compression of the arteries of the arm, there is an immediate stasis (the decision as to whether it happened *post hoc* or *propter hoc* is sometimes difficult, since spontaneous stases often occur). In the enlarged, giant capillaries, in which the flow before compression was very slow and sluggish, and in which there were frequent, spontaneous stases, we often observe that after compression this flow remains visible for a long time before it finally stops. Time intervals of more than one minute are not rare. (The experiment should not be repeated immediately; one must wait several minutes, otherwise the results will be incorrect.) As is to be seen from these contradictory findings under the seemingly identical conditions, we are concerned with a complicated function which apparently represents the resultants of various factors influencing the reflux time. This will be discussed later.

CRITICAL CAPILLARY PRESSURE

Observations of the reappearance of the capillary flow after compression show varying results in different cases. In several examinations of the same case at different times there is, for the most part, a rather constant pressure at which the flow reappears. The experiments must not be done immediately after one another; a rest of several minutes must be interposed. Those patients in whom the pressure at which the flow reappears varies on different examinations seem to belong to that group in which the blood pressure also changes frequently. In general, the capillary flow reappears normally at a pressure of about 10 to 25 mm. below the systolic blood pressure. A striking and abnormal finding was

Difference Between Systolic Pressure and Critical Capillary Pressure

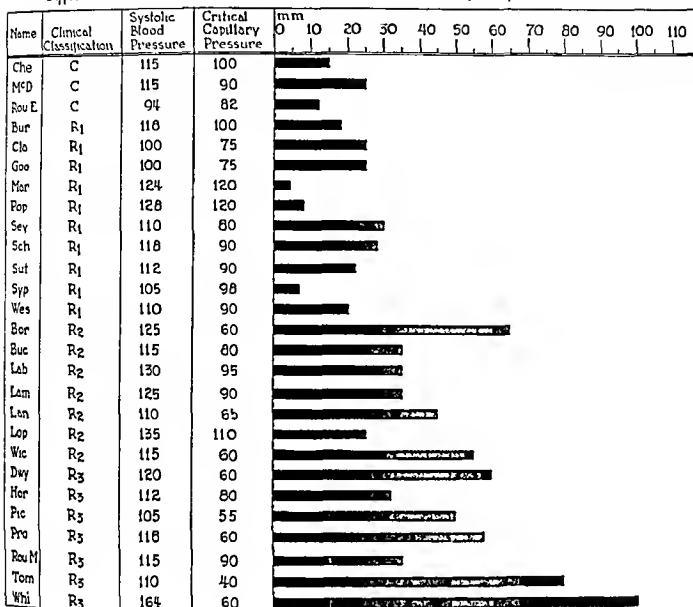


Chart 1.

Difference Between Systolic Pressure and Critical Capillary Pressure

O = before operation

< = after operation

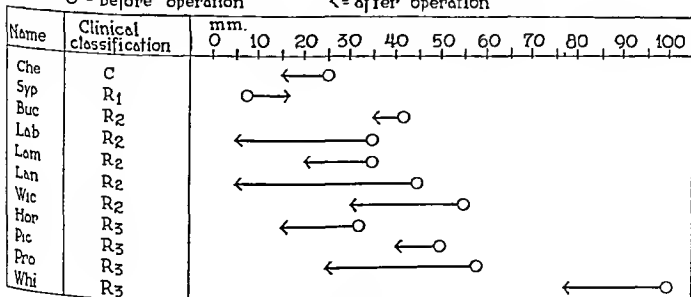


Chart 2.

TABLE I
CHIEF CAPILLARISCOPIC FINDINGS

NAME	CLINICAL CLASSI- FICATION	OPERATION	SLUGGISH FLOW	GIANT CAPIL- LARIES	RESUMPTION OF FLOW 30 MM. OR MORE BELOW SYSTOLIC PRESSURE
Cla	C	R-	+	-	-
		L-	+	-	-
Fre	C	R-	+	-	.
		L-	-	-	.
Rou. E	C	R-	+	-	-
		L-	.	.	.
Bur	R ₁	R-	-	-	-
		L-	.	.	.
Chc	R ₁	R-	-	-	-
		L-	.	.	.
Cron	R ₁	R+	+	-	.
		L+	+	-	.
Goo	R ₁	R+	-	-	-
		L+	+	-	+
McD	R ₁	R+	-	-	-
		L-	-	-	-
Mar	R ₁	R-	-	-	-
		L-	.	.	.
Pop	R ₁	R-	+	-	-
		L-	.	.	.
Sey	R ₁	R-	+	-	.
		L+	.	.	.
Sch	R ₁	R+	-	-	.
		L+	.	.	.
Sut	R ₁	R-	+	-	+
		L-	.	.	.
Syp	R ₁	R-	+	+	-
		L-	.	.	.
Wes	R ₁	R+	+	-	-
		L+	+	.	+
Bor	R ₂	R+	+	+	+
		L+	.	.	+
Buc	R ₂	R-	+	-	+
		L-	.	.	+
Lab	R ₂	R-	+	-	.
		L-	.	.	+
Lam	R ₂	R-	+	+	+
		L-	+	+	.
Leh	R ₂	R-	+	+	.
		L-	.	.	.
Lop	R ₂	R-	-	-	+
		L-	-	-	-
McG	R ₂	R+	+	-	-
		L+	-	-	.
McI	R ₂	R+	-	+	.
		L+	-	+	.
Sir	R ₂	R+	.	+	+
		L+	.	.	.
Wie	R ₂	R-	-	-	.
		L+	.	+	-
Dwy	R ₃	R-	+	+	.
		L+	-	+	.
Gal	R ₃	R+	-	+	.
		L-	+	+	-
Hor	R ₃	R-	+	+	-
		L-	-	+	-

R = Raynaud's disease.

C = Causalgia.

TABLE I—CONT'D

NAME	CLINICAL CLASSIFICATION	OPERATION	SLUGGISH FLOW	GIANT CAPILLARIES	RESUMPTION OF FLOW 30 MM. OR MORE BELOW SYSTOLIC PRESSURE
Lan	R ₂	R- L-	+ +	+ +	+ +
McC	R ₂	R+ L-	- +	- -	- -
Pic	R ₂	R- L-	+ .	+ .	+ .
Pro	R ₂	R- L-	+ .	- .	- -
Rou. M	R ₂	R+ L+	+ +	+ -	- -
Tom	R ₂	R+ L+	+ .	+ .	+ .
Whi	R ₂	R- L-	+ .	+ .	+ .

obtained in severe cases of Raynaud's disease and scleroderma in that the pressure often had to be reduced 60 mm. below the systolic pressure before the capillary flow reappeared. Reappearance of the flow at a point 35 mm. or more below the systolic pressure seems to be due to a local disturbance in capillary function.

In Charts 1 and 2 this relationship is graphically presented. In Chart 1 the difference between systolic and critical capillary pressure is shown in comparison with the severity of the illness; in Chart 2 the differences before and after operation may be seen.

CAPILLARY PERMEABILITY

As mentioned above, in severe cases one frequently may see blood extravasations. This is to be regarded as a sign of increased capillary permeability. Moreover, in a few cases with giant capillaries, we were able to observe the following phenomenon: After the blood pressure cuff was pumped up, a light-refracting substance gradually began to cover these giant capillaries, and the entire field became hazy. This may have been due to a filtration of plasma through the capillary wall because of increased capillary permeability. The criticism that this phenomenon may be explained by a difference in the refraction of light by the giant capillaries lying beneath can be refuted by the fact that we had the opportunity to observe the appearance of this "shiny substance" after prolonged compression in cases in which it was not formerly present. It is, therefore, due to the passage of plasma substance through the capillary wall. In a subsequent examination at another time the capillary picture was again distinct. This may be considered as further proof in favor of our interpretation.

Patients whose fingers were edematous usually showed a vague, hazy, capillary picture. More often it was seen that the originally clear capillary picture became vague and hazy upon prolonged compression, indicating increased permeability. Capillary permeability and its correlation to adrenergic and cholinergic reactions was investigated by the autonomic skin test.¹⁰

CLINICAL AND CAPILLARY GROUPING

In Table I the results of the capillaroscopic examination are compared with the classification of the cases from the clinical and surgical viewpoints. The characteristics which are significant and particularly suitable for a tabular survey are as follows:

1. The presence of giant capillaries (noted in the table by +).
2. Sluggishness of flow (noted in the table by +).
3. The reflux time and the critical capillary pressure (see Method). If the difference between the systolic blood pressure and the critical capillary pressure is above 30 mm., this is noted in the table by +; a - denotes that the difference is less, and a dot that the experiment was not done.

The clinical grouping, from the surgical viewpoint, is as follows:

1. Group 1 of Raynaud (R_1): no obvious local changes on ordinary examination of the affected extremity.
2. Group 2 of Raynaud (R_2): obvious local changes.
3. Group 3 of Raynaud (R_3): obvious local changes and x-ray abnormalities such as bone atrophies.
4. Causalgia (C): pain a prominent symptom with secondary vasomotor changes.

In Table I we see that the plus signs tend to form a group which includes those patients who were also designated as group R_2 or R_3 clinically, whereas the groupings R_1 (mild cases) and C (so-called cases of causalgia) were frequently characterized in the table by minus signs. Insofar as we examined these patients preoperatively, these findings are noted in the table by minus signs. Those patients who were seen only after operation are noted in the table by plus signs. It may be concluded from experience with cases seen before and after operation that the above cases may also have had a pathologic picture before operation, i.e., giant capillaries and sluggish flow.

A comparison of the capillaroscopic and clinical groupings of Raynaud cases revealed certain discrepancies. For example, the nailfolds of Lop and Wic (Table I) showed less marked findings capillaroscopically than we would expect on the bases of the clinical classification, whereas Dwy and Goo showed more severe capillaroscopic findings. The past history of Lop reveals, however, that the signs and symptoms of the disease were localized only in the feet, never in the hands which were always free. She was placed in group 2 only on the basis of the findings in her legs, not in her hands. The patient actually had had only a lumbar sympathectomy. The seeming inconsistency in the classification is thereby explained.

As mentioned above, the capillaroscopic findings in patient Wic were those of a benign case, whereas the clinical impression was that of a moderately severe case. This case is here described in greater detail because of its other interesting features.

CASE REPORTS

CASE 1.—The patient was a young woman, aged 26 years, who impressed the physicians by her persistent complaints of pain in the hands and occasional blanching of the fingers. This was the reason for placing her in group 2, although according to the capillaroscopic exam-

ination she belonged in group 1. Psychologically, she had the personal characteristics of an obsessional neurotic. She showed interesting changes in the capillary picture upon repeated examinations at different times.

She was examined for the first time four months after a left-sided preganglionic sympathectomy. The left hand was dry and warm, the right hand moist, cool, and purple. Only the right hand was examined capillaroscopically at that time. There were signs of a developmental disturbance of the capillaries (expressed in the persistence of the subpapillary plexus and in visible anastomoses with the papillary capillaries) and a flow of moderate speed, a condition usually not found in moderately severe cases of Raynaud's disease.

When the patient was examined eight months after operation on the left hand and four months after operation on the right hand, she was still under the influence of a recent gynergen injection. She said that she was in the habit of taking these injections originally because of asphyxia of the finger, but now because of migraine; she claimed that these injections helped her. Her hands were cold, dry, and showed purplish spots when she entered the room. The most striking capillaroscopic finding was a very marked sluggishness of flow; often there was a complete lack of motion in the microscopic field.

Without being asked, but quite spontaneously, the patient gave the following information: Before the sympathectomy, whenever she had a gynergen injection, the hands became reddish, warm, and moist; since the operation, after a gynergen injection, her hands always became cold and purplish.

One month later the patient was again examined; she had had no gynergen injections for several days. The hands were reddish pale; the palms were colored bright red and were dry and warm. Capillaroscopically there was a rapid blood flow in the capillaries. In contrast to the former findings, the stream never became sluggish and no periods of stasis appeared. The capillaries which, preoperatively as well as at the time of the first postoperative examination, without being under gynergen influence, were found to be dilated, now had a normal lumen. Thus these findings show that the gynergen injection before and after operation had a different effect on the appearance of the hands and of the capillaries.

The influence of gynergen on the asphyxia of the fingers before operation is comprehensible, because ergotamine paralyzes the sympathicus and, therefore, in cases of Raynaud's disease with spastic, constricted arterioles, there is dilatation of the arterioles and thereby a better blood supply to the hands. As a consequence of this the hands become warmer and redder. After sympathectomy the sympathetic action on the arterioles is absent, and the gynergen action has lost its pathway. Nevertheless, as the capillaroscopic examination reveals, it must still have an effect on the capillaries.

According to Cannon⁶ and to our own findings, the sympathectomized zones are hypersensitive to adrenalin. Narrowing of the capillaries, which we found in most cases after sympathectomy, could be explained on this basis. There also seems to be an increased sensitivity to adrenalin-inhibiting substances, such as shown by the gynergen effect postoperatively. Capillaroscopic examination shows that after gynergen injection there is a dilatation of the capillaries in the sympathectomized region. Since this occurs without sympathetic regulation of the width of the arterioles, the consequence of the sudden, marked dilatation of the vessels is a slowing of the flow to the point of stasis. The cyanotic, blue-red color of the hands and the cool temperature are a result of this widening of the capillaries and the slowing of the flow.

The correctness of this assumption is confirmed by the third examination of the patient at a time when she had not received a gynergen injection. The findings were the same as in other sympathectomized patients: narrow capillaries, marked rapidity of flow, and warmth of the hands.

To summarize, the functional tests of the capillaries in the compression test in this patient may be discussed. Before operation on the right side (the left dorsal sympathectomy had been performed four months earlier) the reflux time was seventy-three seconds and the critical capillary pressure was 60 mm., that is,

55 mm. below systolic pressure (115 mm.). On examination of the right side four months after operation and while under the influence of ergotamine tartrate (gynergen), the reflux time in the right hand was thirty-five seconds and the critical capillary pressure was 80 mm., that is, 20 mm. below the systolic pressure of 100 mm. In the left arm, on which an operation was performed eight months previously, the reflux time was forty seconds and the critical capillary pressure was 84 mm., or 16 mm. below the systolic pressure.

One month later, without the influence of gynergen, the reflux time was twenty-five seconds on the right side and the critical capillary pressure was 90 mm., or 28 mm. below the systolic pressure (118 mm.).

Thus we see that the pathologic increase of the reflux time present before operation is significant, that postoperatively, with the patient under the influence of gynergen, it is less noticeable, and that without gynergen the reflux time is almost normal.

The variations in the critical pressure correspond to those in the reflux time. The pathologically low critical capillary pressure becomes distinctly higher after operation and, postoperatively, under the influence of gynergen, it is not so high as without gynergen. But since the blood pressure after the gynergen injection was only 100 mm., the difference between the systolic pressure and the critical capillary pressure ($100 - 80 = 20$ mm.) seemed somewhat less after the gynergen injection than later without gynergen ($118 - 90 = 28$ mm.).

CASE 2.—In the case of Goo there is also a discrepancy between the clinical picture and the capillaroscopic findings in the left hand. Clinically she was placed in group R₁, whereas the capillaroscopic findings were those of a more severe case.

She was a 33-year-old white, single woman, of slender build. Her skin was pale, her hands were cool and pinkish. On October 6, 1939, the date of examination, her pulse rate was 78 without any respiratory arrhythmia. Her blood pressure was 108/72.

Capillaroscopic examination of the right hand revealed a straight corium. There were 8 capillaries per field in the first row, irregular in form, most of them hairpin-like, but short, and some were half figure-of-eight shaped and widened. The width of the vessels changed during examination, some disappearing entirely. There was no marked difference between the arterial and venous limbs of the capillaries. The stream is slow and granular, and in some capillaries is invisible.

The second row reveals some hairpin-like, short, very widened capillaries. Others are dendritic and pointed. The subpapillary plexus is not visible.

Functional test: The blood stream stops after twenty-eight seconds, at a pressure of 130 mm. Hg., and starts again at 70 to 80 mm. Hg.

Capillaroscopic examination of the left hand reveals a straight corium. In the first row there are 6 widened, parallel capillaries, some hairpin-like, some twisted and dendritic. The venous limb is slightly wider than the arterial. The stream is sluggish and granular. In the second row the capillaries are diffusely located, some running horizontally. Some are very widened, sacklike shaped; some are short, some have biscuit forms. The subpapillary plexus is not visible; no archicapillaries can be seen.

Functional test: The stream stops at a pressure of 130 mm. after eight seconds and starts again at 65 mm. In a second test it stops after thirty-five seconds and starts again at a pressure of 55 mm. No retrograde flow is seen during compression.

Summary.—Irregularities in the form and location of the vessels. There are no developmental abnormalities of the capillaries, though there are obviously pathologic processes which lead to regenerative and degenerative changes. There are striking changes of the speed of the blood stream.

The subjective complaints of this patient contrasted strikingly with the objective findings in her hands, although she constantly reiterated that she had pain in her hands and legs. She was a severe obsessional neurotic whose interests were centered on sensations in her hands and legs. She insisted on procedures which gave her only transient relief. This explains the great number of operations which she underwent. They were as follows: at the age of 17, appendectomy; at 20, right ovarian cyst removed; at 31, left lumbar sympathectomy; at 32, in February, 1937, right lumbar sympathectomy; November 5, 1937, left dorsal preganglionic sympathectomy; November 13, 1937, right dorsal sympathectomy; December 16, 1937, left splanchnic resection; December 28, 1937, right splanchnic resection. On October 15, 1938, the patient had a right dorsal sympathectomy, and on November 14, 1938, resection of the fifth to the ninth left intercostal nerves. In spite of all these operations her neurotic complaints continued.

CASE 3.—Another case in which the clinical picture and capillary findings are not in agreement is the patient Dwy. The following are the salient features in this case:

Two and one-half years ago, in October, 1934, the patient crushed his left forefinger tip between two stones. He was seen immediately by his physician and after an x-ray had been taken, was treated conservatively at the local hospital. Later he was x-rayed again by an insurance company. The finger tip had become so sensitive, however, that he was unable to work because of his fear to touch anything with this hand. Two and one-quarter years ago the finger tip was amputated, but the extreme sensitivity remained. Whenever he touched anything, excruciating pain shot up the forearm. His sensitivity contrasted with the objective findings. Two years ago the pain sensation led to the diagnosis of neuroma, and the finger was amputated at the distal interphalangeal joint. No relief was obtained. One and one-half years ago another operation was performed, this time to remove the nerves on either side of the finger. The symptoms remained unchanged in spite of physiotherapy (massage, heat), and the finger seemed to be withering. Nine months ago another amputation was done, this time at the proximal interphalangeal joint, with no change in symptoms. A general physical examination at this time threw no light on the problem, and six "glass boot" treatments produced no change in the symptoms.

The patient has never before noticed numbness or tingling in the extremities or unusual sensitivity to cold. The sensitivity to touch and, more recently, extreme sensitivity to cold is now present in the whole left hand. On exposure to cold air the left hand turns numb and blue and requires ten to fifteen minutes to "thaw out."

On June 16, 1937, a left-sided sympathectomy was performed, with subsequent subjective improvement. The patient was examined for the first time eleven months later. Capillaroscopy at that time revealed in both hands a picture characteristic of a severe case of Raynaud's disease. On re-examination one year later (June 26, 1939) the changes were still more marked in the nonoperated hand. Even at the time of the first examination a large number of giant capillaries with a sluggish flow were found. Other capillaries showed a constantly changing rate of flow in that at times it was rapid and at times sluggish; occasionally it even stopped entirely for prolonged intervals. In addition, complete disappearance and reappearance of the capillaries was often observed. One year ago, in the right, nonoperated arm, the critical capillary pressure was 90 mm., that is, 30 mm. below the systolic blood pressure which was 120 mm. at that time, as it is now. In a follow-up examination on June, 1939, the critical capillary pressure was 60 mm.

From these findings we are justified in concluding that this patient must have had an asymptomatic Raynaud's disease at the time of his original injury. Afterward the accident precipitated a neurosis which revealed itself in his severe sensitivity and in the complicated postoperative course.

These examples show that severe capillary changes may be present in cases without or with inadequate clinical findings. In some cases neurotic complaints are in the foreground and antedate the visible clinical symptoms, since the basic capillary changes are not discovered.

CAPILLAROSCOPY BEFORE AND AFTER SYMPATHECTOMY

Before conclusions concerning the effect of the sympathicus on the capillary flow in Raynaud's disease are drawn from the comparison of capillaroscopic findings before and after preganglionic sympathectomy, the operation, as performed by Smithwick,³¹ is described: "A vertical paravertebral incision centered over the third rib is made. Inner inch of third rib is resected. Second and third intercostal nerves are identified. Inner inch is resected, dividing both anterior and posterior roots, as well as the rami to the sympathetic trunk. The sympathetic nerve is identified and resected below the third ganglion, and the upper end of the section fold is sutured to the insertion of the muscles of the back. The incision is closed with fine silk."

The clinical results of the preganglionic dorsal sympathectomy are as follows: Whereas, before the operation, the affected hand is usually purple and mottled, cold and moist, after the operation it is pale, warm, and dry. It is worthwhile commenting that to the unbiased observer the hand on the sympathectomized side becomes not redder, but paler; and, as mentioned, it is much warmer than before the operation. The difference can be seen most clearly in those patients who have been operated on one side only.

The comparison of the capillaries at the nailfold before and after operation shows changes in the following: (1) the width of the capillaries, (2) the speed of the capillary blood stream, (3) thromboses and blood extravasates, (4) the reflux time, (5) the critical capillary pressure, and (6) the number of visible capillaries.

After sympathectomy in cases of Raynaud's disease a distinct *change in width* of the capillaries could be observed. In the mild cases the abnormal width of the capillaries was decreased. Of the two most severe cases with giant capillaries, in one the capillaries narrowed conspicuously, in the other they did not change; of 6 cases with widened capillaries, 5 showed narrowing after operation (Chart 3). Of the whole group of 15 operated cases, 9 showed narrowing after operation.

The most striking change after operation is, however, the increased *speed of the blood flow in the capillaries*. Of the 11 cases examined for speed of flow before and after operation, 2 showed no change and 9 showed a more rapid flow (Chart 4). In 3 others, examined only after operation, but where comparison could be made with the unoperated hand, all 3 operated cases showed increased flow. The changes are best seen in the mild and moderately severe cases. In the most advanced cases also, even after operation, a sluggish flow is still present in the markedly dilated capillaries, but one sees clearly the tendency to increased speed of the blood flow in the relative rarity and brevity of the periods of stasis as compared to the behavior before operation.

In two cases (Lam, Syp) on re-examination a few weeks after operation, *thromboses and hemorrhagic exudates* could no longer be found, although these were seen before operation.

In one case (Pic) where the increased capillary permeability before operation was characterized by the light-refracting substance in the neighborhood of the giant capillaries, such a "shiny" substance was not present after operation

Average Width of Capillaries*

O = before operation < = after operation

Nome	Clinical classification	-1	1	2	widened	giant
Che	C	⊙				
McD	C		⊙			
Sey	R ₁			← ⊙		
Syp	R ₁		←	⊙		
Buc	R ₂	←	⊙			
Lab	R ₂			← ⊙		
Lom	R ₂			⊙		
Lan	R ₂			←	⊙	
McG	R ₂		⊙			
Wic	R ₂		←	⊙		
Dwy	R ₃				⊙	
Hor	R ₃			←		⊙
Pic	R ₃					⊙
Pro	R ₃			←	⊙	
Whi	R ₃		←	⊙		

*The numbers -1, 1, etc., signify values of less than -1, 1, etc., red blood cells

Chart 3

Speed of Capillary Stream Before and After Operation

O = before > = after

Name	Clinical classification	slow	normal	fast	very fast
Che	C			⊙ →	
Sey	R ₁	⊙ →			
Buc	R ₂	⊙ →			
Lab	R ₂	⊙ →			
Lom	R ₂	⊙			
Lan	R ₂	⊙ →			
Wic	R ₂	⊙ →			
Hor	R ₃		⊙ →		
Pic	R ₃	⊙			
Pro	R ₃	⊙ →			
Whi	R ₃		⊙ →		
Examination only after operation					
non-op. hand = O op. hand = >					
McD	C		⊙ →		
McG	R ₂		⊙ →		
Dwy	R ₃	⊙ →			

Chart 4.

and did not appear until after prolonged compression. This suggests a decrease in capillary permeability.

In the same patient it was also noted that the capillaroscopic picture, which had been very hazy before operation, became much clearer postoperatively. This was in obvious connection with the visible decrease in the swelling of the finger, i.e., with the decrease in the free fluid in the tissue. In addition, the dilatation of the excretory ducts of the sweat glands, which was present preoperatively, was no longer seen.

Capillaroscopic observations of sympathectomized Raynaud's disease have been published by Adson and Brown.¹ The authors discuss a case of Raynaud's disease which had a "resection of the sympathico-cervico-thoracic and second thoracic ganglion and the intervening trunk" on one side. They described the improved capillary flow, the brighter color of blood, and the narrowness of the capillaries on the operated side. The latter they attribute to increased tonus of the capillaries.

Change in the reflux time after operation varies, depending on its preoperative value and on the time which has elapsed since operation. In the group with decreased reflux time were cases with stases occurring after a few seconds as well as those with instantaneous stases. It was shown that the reflux time in these cases increases after operation. For example, in one patient (Syp), the reflux time before operation was only six seconds. On examination two weeks after operation the reflux time was seventy seconds, three weeks after operation, fifty-five seconds, and five weeks after operation, twenty-five seconds. After six weeks there was again a decrease in the reflux time, so that immediately after pumping up the blood pressure cuff there was a stasis in the capillary stream. Three months after operation the reflux time reached forty-five seconds, and six months afterward it was about the same, thirty-nine seconds.

In another patient (Whi) who showed a short reflux time of five seconds preoperatively, the same value was obtained on the fourth day after operation. On the fifth day the reflux time was 52 seconds, on the twelfth, fifteen seconds. However, this was observed three days after an operation on the other arm and the reflux time was fifteen seconds in both extremities.

In patient Che, who showed an immediate stasis upon compression before operation, the reflux time was eight seconds eleven days after operation.

In another patient (Lan), with a decreased reflux time on the right side to four seconds and on the left to five seconds, examination of the left hand five days after operation showed a reflux time of nine seconds, and sixteen days after operation, examination of the right hand showed a value of fifteen seconds.

Finally, in patient Lab, in whom, before operation, there was an immediate stasis of the capillary flow upon pumping up the cuff, the reflux time in the right arm was thirty-seven seconds one week after operation and eighteen seconds in the left arm two weeks after operation.

In some cases with a low reflux time no changes therein appeared during the first four days postoperatively, but the increase is first seen shortly thereafter.

This condition is particularly noteworthy because between the fourth and fifth days there is frequently also an unusual change in the appearance of the hand. Usually, during the first four days after operation, as Smithwick²¹ has often emphasized, the hand is still cold and moist, while on the fifth day it becomes warm and dry.

The curve for the reflux time in the above-mentioned case (Syp), as in other cases, shows that the increase in time which appears a few days after operation gradually decreases in the course of the following few weeks, but that later a value is established which does not change in the following few months and seemingly may be regarded as constant.

The values of the reflux time obtained for patient Whi create the impression that the operation on one side influences the postoperative results on the other side, since the reflux time in one arm, increased because of the operation, decreases at the moment a sympathectomy is performed on the other side.

In summary, it may be said that in cases of Raynaud's disease with lowered reflux time there is an increase after operation.

In discussing those cases with an increased reflux time before operation, it must be stated that in the majority they are severe cases. Here we see the opposite of what we saw in cases with decreased reflux time. After operation there is a significant decrease in reflux time, the value sometimes sinking below normal. Patient Pic, who was examined several times before operation, showed a markedly increased reflux time in her enormously dilated capillaries. However, it was striking that before operation a stasis could occasionally be seen immediately following compression. This, however, was not always due to compression, but was one of the frequent spontaneous stases which were characteristic of this case. These spontaneous stases ceased after operation. Sixteen days after operation the reflux time was fifteen seconds. Two and one-half months after operation the afterflow time in the right arm was twenty-six seconds, in the left, three months after operation, it was five seconds.

Another patient (Hor) who, before operation, had a reflux time of ninety seconds in the right hand and of sixty-five seconds in the left hand, showed a reflux time of eight seconds on the left hand fifty-one hours after operation on this side, and of five seconds on the fourth day after operation. On examination two weeks after left-sided preganglionic dorsal sympathectomy and one week after right-sided preganglionic dorsal sympathectomy, the reflux time in the right hand was twenty-five seconds.

In patient Pro, who, according to the clinical picture and the capillaro-scopic findings is one of the severe cases of Raynaud's disease, a reflux time of thirty-seven seconds was found before operation. Three days after left-sided preganglionic dorsal sympathectomy and twelve days after right-sided preganglionic dorsal sympathectomy the reflux time was thirty seconds on the left and thirty-five seconds on the right. Four days later, in the left hand, the reflux time was twelve seconds; in the right hand varying results were obtained in variously formed capillaries of different widths. In a narrow capillary a time of twelve seconds was found and in a widened giant capillary, thirty-two seconds, while in the deeper layers values of five seconds and eight seconds were obtained.

In general, it may be said that in cases with a pathologically increased reflux time, a sudden and excessive fall thereof occurs immediately after operation. Then its value gradually approaches a normal level.

In some patients with preoperatively prolonged reflux time in whom three or more months have passed since operation, findings of thirty-five to forty-five seconds reflux time have often been obtained, i.e., a moderately prolonged reflux time. This finding was obtained in 13 of 25 hands which have been examined to date. In few patients in whom there was no retrograde flow in the compression experiment, a reversal in the direction of flow was seen upon compression after operation.

The *critical capillary pressure* has a distinct relation to the systolic blood pressure. If the blood pressure cuff is pumped over the systolic pressure and kept at this level, no blood can flow through the arterioles and reappearance of the capillary flow is impossible. Those few cases which, paradoxically, have a critical capillary pressure above the initial systolic pressure can doubtlessly be explained by the fact that they have a markedly fluctuating blood pressure which rose above its initial value during the examination.

If we compare the preoperative and postoperative differences between systolic pressure and the critical capillary pressure, we almost always see that after operation the critical capillary pressure is nearer the systolic pressure than before operation. Of the 11 cases in which this observation was made before and after operation, 10 showed a decrease and one showed an increase (Chart 2).

An interesting phenomenon which we observed in several cases and which was clearly changed after operation is the following: A series of patients who bore the occlusion of the circulation in the compression experiment 1' - 3' without any symptoms, upon gradual release of the pressure, felt a distinctly painful sensation at about the level at which the stream reappeared capillaroscopically. After sympathectomy this pain was no longer elicited.

The number of capillaries per microscopic field visible at the nailfold before and after operation shows no marked variation. Our observations showed no increased number of capillaries such as described by others. In a few cases after operation the first row of visible capillaries was much further from the corium than before operation. This can be explained by the fact that the capillaries of the first row are completely emptied after operation.

Before these findings can be discussed, the correlations between the speed of the capillary flow and the pulse rate should be considered. Such a comparison in our patients shows that there is no direct correlation, but, on the other hand, in cases with slow pulse rates, both rapid and slow rates of flow are found. This did not surprise us, since in patients who had a unilateral sympathectomy we found a sluggish capillary flow contralaterally and a more rapid flow homolaterally. In nonoperated persons with Raynaud's disease who had a rapid pulse rate, we almost always found a sluggish flow on the affected side. Those patients whom we observed with pulse rates over 100 were severe cases of the disease.

This phenomenon might be explained by supposing that the high pulse rate represents a compensatory mechanism to bring about a better flow. One might also bear in mind that, according to various authors, in cases of Raynaud's disease there is an increased activity of the adrenals, and this in turn causes the higher pulse rate. In any case, it shows that capillary circulation is, to a great extent, independent of the main circulation and probably reacts to processes in the tissue.

A simple relationship between systolic pressure and the rate of capillary flow does not exist, since we find rapid as well as sluggish flow in cases with normal pressure. This is apparent in those patients operated on on one side only, since they show a rapid flow on the operated side and a sluggish flow on the nonoperated side.

In the nonoperated patients it was striking that in the group of those with particularly low blood pressure (80 to 110 mm.) the capillary flow was slow in 7 of 9 cases in the group.

DISCUSSION

We can now explain the previously mentioned postoperative pallor and higher temperature of the hand. The former is due to the narrowness of the capillaries which is now present. The latter is due to the increased rate of flow following the operation. One might say, rather, that the temperature of the hand is dependent on the amount of blood flowing through the skin capillaries per unit time, and this is partly dependent on the width of the arteries.

One must not assume therefore, that the acceleration of the flow seen microscopically is a consequence of the small diameter of the capillaries, but, because of the warmth of the hand, one must maintain that despite the narrowness of the capillaries the amount of blood flowing through them at a given time is greater after sympathectomy than before it.

The pallor of the hand observed postoperatively agrees with the findings of Dale and Richards,⁹ who made their observations on the unpigmented cat's paw and saw that the denervated extremity was distinctly warm, but at the same time paler than the pads of the normal foot.

The narrowing of the capillaries after sympathectomy occurs for various reasons. In describing capillary behavior we have avoided the use of the words "spastic" and "atonic" and have restricted ourselves to the words "narrowed" and "widened," in order to emphasize that the change in the width of the capillaries is not indicative of an active movement of the vessels. There is no doubt that the change in the width of the capillaries is not dependent to any great extent on the flow in the arterioles, but is for the most part the result of metabolic changes in the surrounding tissues. Dilatation of the arterial limb is due to a passive distention as a reaction to the environment and swelling of Rouget's cells, whereas narrowing is attributed to inhibition of the surrounding tissue which then compresses the capillaries. Dilatation of the venous limb is more the result of changes in the environment, i.e., of the production of acid metabolites. Since the capillaries have only endothelial layers, no smooth muscle and no connective tissue, the sympathectomy can affect only the arterioles. For this reason, the operation may help in part indirectly: (1) by elimination of the spasm of the arterioles bringing about an acceleration of the blood flow

and a consequent improvement in the local tissue metabolism; (2) through the liberation of a chemical substance (H-substance), the intermediary production of which was inhibited before the nerve fibers were dissected.

The improvement in the capillary condition and the narrowing, therefore, occurs because (a) after the elimination of the spasm the vis a tergo causes an increased rate of flow, (b) the content of the capillaries becomes more mobile, (c) the external pressure decreases due to the changed condition of the tissue, (d) the capillary wall becomes less permeable to the plasma, the blood becomes more dilute, the blood corpuscles less englobated. Each of these factors plays a role.

In consideration of the above-mentioned factors, the postoperative change in the reflux time becomes significant as a functional test. In the cases with sluggish blood flow and very short reflux time, the operation produced an increase in the reflux time because of the reasons discussed, an acceleration of the blood flow, diminution of the tissue pressure, and dilution of the blood. In the few cases with a sluggish flow and a reflux time lengthened because of the tremendous expansion of the capillaries, the operation provoked a relative reduction of the reflux time, particularly by relaxing the arterial spasm and improving the blood flow.

The critical capillary pressure depends on the same factors which affect the reflux time. As a result of the sympathectomy, the critical capillary pressure is nearer the systolic blood pressure, varying directly with the effect of the operation on the basic pathologic factors. The more rapid the capillary flow and the narrower the capillaries, the closer to systolic pressure is the critical capillary pressure.

All these observations show the manifold pathways by which the damaging factors lead to the development of Raynaud's disease. Emotional factors may play a role in the precipitation and in the continuation of these processes, but further study is needed before the importance of this role can be evaluated.

SUMMARY

In this investigation the capillary response was studied in 29 cases of Raynaud's disease.

1. Capillaroscopic observation of cases of Raynaud's disease shows that the disease may be present before the appearance of the clinical symptoms. In these cases abnormal capillaries can be seen when other vasomotor symptoms are not present.

2. In the majority of cases of Raynaud's disease there exists a persistence of the subpapillary plexus and a persistent connection between it and the outgrowing capillaries.

3. Two objective measurements are introduced to test the dynamics of capillary circulation: (a) *reflux time*, i.e., that time which elapses before the capillary flow ceases, when the upper arm is compressed with a blood pressure cuff, pumped up to 30 mm. above the systolic blood pressure; (b) *critical capillary pressure*, i.e., that pressure at which the flow once again reappears when the cuff in the above experiment is gradually decompressed at the rate of 10 mm. per two seconds.

4. Capillaroscopy can define the severity of the disease more accurately than the gross clinical findings.

5. The preganglionic sympathectomy has a distinct influence on the capillary picture of Raynaud's disease: (a) in 12 of 14 cases the speed of the blood flow increased; (b) in cases with a slow reflux time, the time increased, and vice versa; (c) in 10 out of 15 cases the width of the capillaries decreased; (d) the capillary permeability decreased; (e) the dilatation of the excretory ducts of the sweat glands vanished after operation; (f) these changes in the capillary picture after sympathectomy become less distinct in time; (g) the subjective symptoms of the patient are to some extent independent of the clinical picture. Some have the same typical capillary picture as before operation, even though relieved of complaints. Others show objective improvement clinically, but retain their subjective complaints, because a neurasthenia may become apparent, which may have been a part of the picture from the beginning.

REFERENCES

1. Adson, A. W., and Brown, G. E.: Treatment of Raynaud's Disease by Resection of Sympathico-Cervicothoracic and Second Thoracic Ganglions and Intervening Trunk, *J. A. M. A.* 29: 444, 1929.
2. Bishop, G. H., Heinbecker, P., and O'Leary, J. L.: The Function of the Non-myelinated Fibers of the Dorsal Roots, *Am. J. Physiol.* 106: 647, 1933.
3. Bordley, J., Grow, M. H., and Sherman, W. B.: Intermittent Blood Flow in the Capillaries of Human Skin, *Bull. Johns Hopkins Hosp.* 62: 1, 1938.
4. Braeucker, W.: Probleme und Erfolge der Sympathieschirurgie, *Zentralbl. f. Chir.* 63: 1294, 1936.
5. Brown, G. E.: The Skin Capillaries in Raynaud's Disease, *Arch. Int. Med.* 35: 36, 1925.
6. Cannon, W. B.: Factors Affecting Vascular Tone, *Am. Heart J.* 14: 383, 1937.
7. Clark, E. R., and Clark, E. C.: The Relation of the Ruyet Cells to Capillary Contraction, *Am. J. Anat.* 35: 265, 1925.
8. Collier, F. A., and Maddock, S.: Quoted by Pret, M. M., and Kahn, E.: Vasomotor Phenomena Related to Raynaud's Syndrome, *Arch. Neurol. and Psychiat.* 35: 79, 1936.
9. Dale, H. H., and Richards, A. N.: *J. Physiol.* 52: 110, 1918.
10. Duryce, A. W., and Wright, I. S.: Present-Day Technique for the Study of Human Capillaries, *Am. J. M. Sc.* 185: 664, 1933.
11. Ebbecke, U.: *Arch. f. d. ges. Physiol.* 169: 1, 1917.
12. Forbes, H. S., and Cobb, S.: Vasomotor Control of Cerebral Vessels, *Proc. Assoc. Res. Nerv. & Ment. Dis.* 18: 201, 1937.
13. Freeman, N. E., and Zeller, J. W.: Effect of Temperature on the Volume and Flow of Blood Through the Sympathectomized Paw of Dog With Observations on Oxygen Control and Capacity, Carbon Dioxide Content, and pH of the Arterial and Venous Blood, *Am. J. Physiol.* 120: 475, 1937.
14. Griffith, I. A.: The Frequent Occurrence of Abnormal Cutaneous Capillaries in Constitutional Neurasthenic States, *Am. J. M. Sc.* 188: 180, 1932.
15. Heinbecker, P., and Bishop, G. H.: Mechanism of Spastic Vascular Disease, *Ann. Surg.* 107: 270, 1938.
16. Hunt, J. H.: The Raynaud Phenomenon. A Critical Review, *Quart. J. Med.* 5: 412, 1936.
17. Jaensch, W.: Die Hautkapillaroskopie, Halle a.S., 1929, Carl Marhold.
18. Krogh, A.: The Anatomy and Physiology of the Capillaries, New Haven, 1924, Yale Univ. Press.
19. Krogh, A., and Harrop, G. A.: On the Substance Responsible for Capillary Tonus, *J. Physiol.* 54: 125, 1920-21.
20. Lange, F.: Funktionsprüfung der Arterien—mit einer kapillarmikroskopischen Methode, *Arch. klin. med.* 48: 58, 1925.
21. Leader, S. D.: Capillary Microscopy in Children, *Am. J. Dis. Child* 44: 403, 1932.
22. Lewis, T.: Changes in Arteries in Raynaud's Disease, *Clin. Sc.* 3: 287, 1938; Preganglionic Sympathectomy, *Ibid.* p. 321; Vascular Disease, *Arch. Int. Med.* 62: 482, 1938.

24. Lewis, T., and Landis, E. M.: Some Physiological Effects of Sympathetic Ganglionectomy in the Human Being and Its Effect in a Case of Raynaud's Malady, *Heart* 15: 151, 1929-1930.
25. Mittelman, B., and Wolff, H. G.: Affective States and Skin Temperature, *Psychosom. Med.* 1: 271, 1939.
26. Mueller, O.: *Die Kapillaren der menschlichen Koerperoberflaeche in gesunden und kranken Tagen*, Stuttgart, 1922, Ferdinand Enke.
27. Mueller, O., and Parisius, W.: *Die Blutdruckkrankheit*, Stuttgart, 1932, Ferdinand Enke.
28. Pearse, H. E.: Influence of the Heat Regulatory Mechanism of Raynaud's Disease, *Am. Heart J.* 10: 1005, 1935.
29. Raynaud, M.: Nouvelles recherches sur la nature et le traitement de l'asphyxie locale des extremités, *Arch. gén. de méd.* 1: 89, 1874.
30. Simpson, S. L., Brown, G. E., and Adson, A. W.: *Arch. Neurol. & Psychiat.* 26: 687, 1931.
31. Smithwick, R. H.: Modified Dorsal Sympathectomy for Vascular Spasm (Raynaud's Disease) of Upper Extremity. Preliminary Report, *Ann. Surg.* 104: 339, 1936; Value of Sympathectomy, *New England J. Med.* 216: 141, 1937.
32. Smithwick, R. H., Freeman, N. E., and White, J. C.: *Am. J. Physiol.* 107: 529, 1934.
33. Sunder-Plassmann, P.: Zum Raynaud Problem, *Zentralbl. f. Chir.* 65: 994, 1938.
34. Weib, E., and Dieter, W.: Die "Strömung in den Kapillaren und ihre Beziehung zur Gefäßfunktion, *Zentralbl. f. Herz- u. Gefässkr.* 12: 23, 1920.
35. White, J. C.: *The Autonomic Nervous System*, New York, 1935, The Macmillan Co.
36. Wright, I. S., and Duryee, A. W.: Human Capillaries in Health and in Disease, *Arch. Int. Med.* 52: 545, 1933.

THE EXCRETION OF GOLD FOLLOWING THE ADMINISTRATION OF GOLD SODIUM THIOMALATE IN RHEUMATOID ARTHRITIS*

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IT IS generally agreed by those who have had a large experience with gold therapy in rheumatoid arthritis that this form of treatment is followed by encouraging therapeutic results in a large proportion of patients. However, toxic reactions and untoward effects are so common and so serious that there is great urgency for an increase in our knowledge relative to the mode of action, toxicology, and excretion of gold compounds. We have previously reported¹ the marked bacteriostatic power of the serum against many common laboratory organisms which develops following the administration of gold sodium thiomalate. Similar observations were made after the administration of gold sodium thiosulfate and aurothioglucose. These effects may, but do not necessarily, throw some light on the mechanism by which beneficial results are obtained with gold therapy. We recognize that as yet the causative agent of rheumatoid arthritis has not been demonstrated, and that the disease has not been conclusively shown to be of infectious origin.

OBJECTIVE

Our objective here is to report studies on the urinary excretion of gold following the parenteral administration of gold sodium thiomalate. In view of the long duration of unfavorable reactions when they do occur, a special objective was to determine the length of time excretion through the urine con-

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tinued after administration was stopped. Less complete observations were made on the gold content of the synovial fluid, stool, and saliva. We were also interested in determining the amount of gold present in the whole blood and blood serum at varying intervals, and the relationship of these levels to the urinary excretion, but these latter studies will be reported subsequently. All such studies, when coordinated with the occurrence of beneficial effects and toxic reactions, should lead to a clarification of the technique of aurotherapy.

METHOD

The method we used for the determination of urinary gold was described by Pollard² in 1937, and by Jamieson and Watson in 1938.³ Some modifications necessary to make the method applicable to the analyses of materials other than urine will be more completely described in a subsequent paper. Analysis of known samples by these methods resulted in practically 100 per cent recovery. The details observed in the collection of specimens are recorded under the separate experiments described below. Whenever possible the twenty-four-hour urine collections were started at 8:00 A.M., and the gold salt was given as near after 9:00 A.M. as possible. All patients studied were suffering from rheumatoid arthritis and the observations were made during routine treatment with gold sodium thiomalate administered parenterally in aqueous solution. As a rule, no other medication was given. The average dose of gold sodium thiomalate varied from 25 mg. once a week to 50 mg. every other day. The results of our analyses are recorded in terms of metallic gold. It is to be remembered that gold sodium thiomalate contains only 50 per cent metallic gold.

RESULTS

Experiment 1. O. J., female, aged 35 years, with rheumatoid arthritis, received weekly injections of gold sodium thiomalate after two control urinalyses had been done. These, of course, were negative for gold. Subsequently, urines were collected twenty-four hours before and twenty-four hours after the injection of the gold salt. Table I records our results.

TABLE I
URINARY EXCRETION OF GOLD FOLLOWING SUBCUTANEOUS INJECTIONS OF GOLD SODIUM THIOMALATE

CASE	DATE	ADMINISTRATION OF GOLD SALT (MG.)	VOLUME 24 HR. URINE (C.C.)	URINARY EXCRETION AS AU IN 24 HR. (MG.)
O. J., female, aged 35 years	Mar.	14-15	1,125	0
		20-21	925	0
		21-22	840	0.38
		27-28	1,000	0.08
		28-29	935	0.15
	April	3-4	1,125	0.08
		4-5	780	0.30
		10-11	860	0.13
		11-12	810	0.42
		17-18	720	0.23
		18-19	1,060	0.44
		24-25	1,000	0.18
		25-26	770	0.28
	May	1-2	800	0.24
		2-3	700	0.75
		8-9	510	0.32
		9-10	600	0.40

It will be observed that the urinary excretion of gold was less during the twenty-four hours before than the twenty-four hours after the injection of gold salt, but that this difference appeared to decrease week by week.

Experiment 2. A male, aged 38 years, was given injections up to 50 mg. every other day. It will be observed (Table II) that the urinary excretion gradually increased, reaching a maximum of 2.07 mg. During the thirteen days under observation he received a total of 280 mg. of gold salt (140 mg. of metallic gold). He excreted through the urine during this period (allowing an estimated excretion of 0.40 for June 15 to June 16, when the urinary determination was omitted) a total of 12.83 mg. of metallic gold, or roughly 9 per cent of his intake of metallic gold during this period. Serum levels were determined on four days, the results varying from 0.22 to 0.50 mg. per cent. On June 18 the serum content was 0.50 mg. per cent and the synovial fluid content was likewise 0.50 mg. per cent. For three days, starting June 18, carmine-marked stools were collected. It was found that during this three-day period, the patient excreted through the stool only 0.66 mg. It will be observed that the urinary loss during this three-day interval was 4.27 mg. The stool excretion, therefore, was very much less in proportion to the urinary excretion during these three days.

TABLE II

URINARY AND STOOL EXCRETION OF GOLD, AND GOLD CONTENT OF SERUM AND SYNOVIAL FLUID FOLLOWING SUBCUTANEOUS INJECTIONS OF GOLD SODIUM THIOALATE

(Male, Aged 38 Years)

DATE	GOLD SALT (MG.)	VOLUME 24 HR. URINE (C.C.)	AU CONTENT 24 HR. URINE (MG.)	AU CONTENT SERUM (MG. %)	AU CONTENT STOOL (MG. %)	AU CONTENT SYNOVIAL FLUID (MG. %)
June 12-13	5	1,075	0.05			
13-14		910	0.08			
14-15	25	1,050	0.37			
15-16						
16-17	50	2,100	0.55			
17-18		1,200	0.90			
18-19	50	2,200	1.58	0.50	0.66	0.50
19-20		1,475	1.15			
20-21	50	1,600	1.54	0.32		
21-22		1,650	1.23			
22-23	50	1,800	1.67	0.22		
23-24		1,750	1.64			
24-25	50	1,700	2.07	0.44		

Experiment 3. M. W., female, aged 38 years, had received 330 mg. of gold sodium thiomalate previous to the excretion studies we made. Subsequently, 25 mg. of gold salt were given every seven days. Table III records the daily urinary excretion of metallic gold. In this instance, the excretion became relatively fixed, varying between 0.17 and 0.51 mg. in twenty-four hours. From these observations, one might suggest that there was a renal threshold for gold excretion, but the evidence of Experiment 2 (where the excretion gradually increased) tends to contradict this impression.

During the fourteen days from April 26 through May 10 this patient received 50 mg. of gold salt or 25 mg. of metallic gold. She excreted through the urine during this period (assigning an estimated average of 0.33 mg. for April 27 when the determination was omitted) a total of 4.35 mg. of gold, or 17.2 per cent of the intake. This proportion is larger than that found in Experiment 2 where the percentage of excretion was 9 per cent.

Experiment 4. It was important to know how long the urinary excretion of gold continued after the administration of gold salt had been discontinued. Table IV records the gold content of the urine at varying days from 60 to 300 after the cessation of therapy. In Case 5 there was 0.04 mg. of gold in the urine on the 210th day, 0.03 mg. on the 240th day, but no gold on the 270th day. In Case 2, 0.01 mg. was found on the 300th day. All observations made show that gold is still excreted long after the administration has been discontinued.

TABLE III

URINARY EXCRETION OF GOLD FOLLOWING SUBCUTANEOUS INJECTIONS OF GOLD SODIUM THIOALATE

CASE	DATE	ADMINISTRATION OF GOLD SALT (MG.)	VOLUME 24 HR. URINE (C.C.)	URINARY EXCRETION AS Au IN 24 HR. (MG.)
M. W., female, aged 38 years	Dec. 20, 1939 to Apr. 26, 1940	330		
	Apr. 25-26		370	0.36
	26-27	25.0	300	0.51
	27-28		-	-
	28-29		690	0.24
	29-30		880	0.25
	30-May 1		900	0.17
	May 1-2		750	0.35
	2-3		870	0.34
	3-4	25.0	1250	0.24
	4-5		500	0.47
	5-6		250	0.42
	6-7		825	0.42
	7-8		800	0.36
	8-9		775	0.34
	9-10		360	0.24
	10	25.0		

TABLE IV

URINARY EXCRETION OF GOLD AFTER STOPPING THE ADMINISTRATION OF GOLD SODIUM THIOALATE FROM 60 TO 300 DAYS

CASE	NO. DAYS SINCE LAST DOSE	LAST DOSE (MG.)	TOTAL GOLD SALT GIVEN	VOLUME 24 HR. URINE (C.C.)	URINARY GOLD EXCRETION (MG.)
1	60	25	340	1,500	0.13
2	300	10	340	1,200	0.01
3	102	10	493 (second course)	1,000	0.16
4	90	30	575	840	0.16
5	210	25	200	530	0.04
	240	25	200	1,200	0.03
	270	25	200	1,675	0.00
6	135	50	1,100	1,400	0.09
7	100	30	410	860	0.10
8	98	50	1,005	1,370	0.17
9	90	10	85	1,650	0.08
10	210	25	1,000	1,000	0.09

TABLE V

EXCRETION OF GOLD BY THE STOOL

CASE	TOTAL GOLD SALTS GIVEN (MG.)	LAST DOSE (MG.)	NO. DAYS SINCE LAST DOSE	NO. DAYS STOOL STUDIED	TOTAL STOOL EXCRETION Au (MG.)	AVERAGE DAILY URINE EXCRETION Au (MG.)	AVERAGE DAILY STOOL EXCRETION Au (MG.)
1	355	25	One	1	0.64	?	-
2	285	50	Second day	3	1.94	?	0.65
3	180	50	First and third days	3	0.66	1.42	0.22

TABLE VI
EXCRETION OF GOLD BY THE SALIVA

CASE	AMOUNT OF GOLD SALTS GIVEN (MG.)	LAST DOSE (MG.)	NO. DAYS SINCE LAST DOSE	VOLUME OF SALIVA (C.C.)	AU CONTENT (MG.)
1	480	50	2	100	0
2	500	50	3	56	0

TABLE VII

URINARY EXCRETION OF GOLD FOLLOWING SUBCUTANEOUS INJECTIONS OF GOLD SODIUM THIOMALATE, AND THE EFFECT OF SODIUM BICARBONATE ON THE URINARY EXCRETION
(Female aged 35 years)

DATE	GOLD SALT (MG.)	AU CONTENT 24 HR. URINE (MG.)	MEDICATION SODIUM BICARB. (DRAMS O.D.)
June 27	10		
28			
29	25		
30			
July 1	50		
2			
3	50		
4			
5			
6	50		
7			
8	50		
9			
10	50		
11			
12			
13	50		
14	Slight dermatitis, both axillae, gold salt discontinued		
15		0.65	
16		0.71	
17		0.96	
18		0.39	
19		0.58	
20		0.68	
21		0.50	
22		0.53	1
23		0.53	2
24		0.38	4
25		0.44	4
26		0.53	4
27		0.35	4
28			4
29			4
30			4

Experiment 5. A few observations were made on the metallic gold content of the stool. Stools were appropriately marked with carmine in each case. It will be observed (Table V) that the excretion of gold through the stool varied between 0.22 and 0.65 mg. in twenty-four hours.

Experiment 6. In two instances (Table VI) saliva was collected during the course of gold sodium thiomalate administration. In neither of these instances was metallic gold found in the saliva.

Experiment 7. S. P., female, aged 35 years, had never received gold before the institution of these observations. After she had received 330 mg. of gold salt, she developed a slight dermatitis under both axillae, which may or may not have been due to gold. However, the gold administration was discontinued. Subsequently from July 17 through July 29, the

daily urinary excretion was determined (Table VII). In this case, as in Experiment 3, the urinary excretion seemed rather fixed. After the seventh day of this relative fixation of excretion, we were interested in observing the effect of sodium bicarbonate on the rate of excretion. Up to 15.6 Gm. were administered by mouth daily. It will be observed that from the eighth to the thirteenth day, the urinary excretion continued relatively constant, the administration of sodium bicarbonate apparently having no effect in this respect.

Our object here was to observe whether sodium bicarbonate had the same accelerating effect on the excretion of gold as it has been found to have on the excretion of lead. These studies are being extended and will be reported more fully at a later date.

CONCLUSIONS

1. Following the subcutaneous administration of gold sodium thiomalate in patients with rheumatoid arthritis, metallic gold can be consistently recovered from the urine, and the twenty-four-hour excretion determined.

2. In two instances the urinary excretion of gold approximated 9 and 17.2 per cent of the intake of gold during the period of observation.

3. Gold was found in the urine from 60 to 300 days after administration of gold sodium thiomalate had been stopped.

4. The gold content of the stool in three instances ranged from 0.22 to 0.65 mg. in twenty-four hours.

5. No gold could be recovered from the saliva in the two instances studied.

6. As far as our observations were extended, the administration of sodium bicarbonate by mouth had no quantitative effect on the excretion of gold in the urine.

REFERENCES

1. Hartung, E. F., and Cotter, J.: The Effect of Gold Sodium Thiomalate Administration on the Bacteriostatic Properties of the Serum in Patients With Rheumatoid Arthritis, *J. LAB. & CLIN. MED.* 26: 1274, 1941.
2. Pollard, W. B.: The Micro-determination of Gold, *Analyst* 62: 507, 1937.
3. Jamieson, A. R., and Watson, R. S.: The Determination of Minute Quantities of Gold in Urine, *Analyst* 63: 702, 1938.

STUDIES IN BACTERIOPHAGE VI

THE EFFECT OF SULFAPYRIDINE AND SULFANILAMIDE ON STAPHYLOCOCCI AND *B. COLI* AND THEIR RESPECTIVE BACTERIOPHAGES*

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INTRODUCTION

IN A PREVIOUS publication¹ we reported the effect of a large series of anti-septics, used in surgical practice, on staphylococcus, *E. coli*, and *B. pyocyaneus* bacteriophages. We found that sulfanilamide (prontosil and prontylin) and its derivative, sulfapyridine, were the only substances tested which exerted no significant influence on the lytic phenomenon.

In a number of later experiments with weaker phages, these drugs seemed to have an adjuvant effect upon the action of bacteriophage, but they had no appreciable effect on phages of high titer and lytic ability. This suggested that the combination of phage and one of these drugs may be used in those cases in which the causative organism was partially resistant to phage or in mixed infections where each may have a specific action.

EXPERIMENTAL TECHNIQUE

Our stock mixture of staphylococcus phages S1, and individual phages 43† and BH‡ were tested against 50 strains of staphylococci obtained from various sources and possessing various degrees of phage susceptibility, with and without sulfapyridine and sulfanilamide.

Our stock mixture of *E. coli* phages B4 and individual phage MK were likewise used in conjunction with these two substances when propagated against 45 strains of *E. coli* of various degrees of susceptibility.

The technique of the experiments was in general the same as in our previous work.¹ All lysis tests were carried out in 5.5 c.c. of Savita broth. Plating of lysed tubes on 5 per cent sheep's blood agar indicated the presence or absence of surviving bacteria. The titer of the phages was determined by making decimal dilutions in saline, adding bacteria and then counting the plaques when these dilutions were transplanted and incubated on 1 per cent plain agar plates. Generally, in carrying out phage susceptibility tests, 0.05 c.c. of staphylococcus phage, or 0.25 c.c. of *E. coli* phage, were used in 5.5 c.c. of Savita broth, together with approximately 50 to 60 million bacteria per cubic centimeter. Variations from this technique are indicated in certain experiments.

Sulfapyridine and sulfanilamide were used in a 10^{-4} dilution (1:10,000), which is about the highest concentration which these substances ordinarily reach

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†Obtained in 1933 from Dr. Bulgakov.

‡Obtained in 1933 from Dr. Gratia.

in the circulation of treated patients. In order to obtain the necessary concentration, 0.5 c.e. of the 10^{-3} dilution (1:1,000) of these drugs in Savita broth (sterilized by boiling for five minutes) was added to 5 c.e. of Savita.

RESULTS

A. The Effect of Sulfapyridine (SP) and Sulfanilamide (SN) on the *Staphylococcus Bacteriophage* Phenomenon

I. *The Degree and Permanency of Bacteriolysis Both With and Without SP and SN.*—S1 phage (slightly weakened by forty-eight-hour standing at room temperature) was set up with 15 strains of staphylococci, most of which were somewhat resistant to the phage. Two series of tubes containing 5.5 c.e. of Savita plus sulfapyridine SP (1:10,000) were employed. One series was inoculated with 0.05 c.e. of phage and 0.1 c.e. of an eighteen-hour Savita broth culture of bacteria (making about 50 million per cubic centimeter), and the other series with bacteria alone. Two series of 5.5 c.e. of Savita without sulfapyridine, inoculated in a similar manner, served as controls. The results of the test are shown in Table I. It is seen that after four and a half hours' incubation,

TABLE I

	TIME	CULTURES														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Lysis by phage	4½ hours	-	4+	4+	3+	3+	3+	-	4+	1+	4+	4+	4+	4+	4+	1+
S1 in Savita	22½ hours	-	1+	4+	-	4+	4+	1+	4+	4+	4+	4+	4+	4+	4+	3+
	5 days	-	-	2+	2+	4+	4+	-	-	4+	4+	1+	4+	-	4+	2+
Lysis by phage	4½ hours	-	4+	4+	4+	4+	3+	1+	1+	2+	4+	4+	8+	4+	3+	1+
S1 in Savita	22½ hours	-	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
+ SP	5 days	-	4+	4+	4+	4+	4+	2+	4+	4+	4+	4+	4+	4+	4+	4+
Bacterial control in Savita	4½ hours	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	22½ hours	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bacterial control in Savita	4½ hours	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
+ SP	22½ hours	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

4+ = Complete clearing.

4± to ± = Different degrees of clearing.

- = No lysis.

tion, the degree of clearing or growth in corresponding tubes with and without SP was essentially the same. After twenty-two and a half hours' incubation, four cultures, only partially clear in the absence of sulfapyridine, were completely clear in the corresponding tubes containing sulfapyridine. After five days' incubation five cultures which were cloudy in the absence of sulfapyridine were completely clear (4+) in the corresponding tubes containing sulfapyridine, and two others, which in the absence of SP showed 2+ lysis, were almost clear (4±).

The experiment was repeated with the individual phage No. 43 and eight strains of staphylococci, and similar results were obtained. This phage produced complete visible lysis of all these strains in the test tubes without SP or SN in twenty-four hours, but five of the cleared cultures yielded growth upon plating and these tubes became cloudy within seventy-two hours. The corresponding tubes containing phage and either SP or SN not only became clear, but also gave no growth upon plating, and remained clear for fifty days at room temperature when examined last.

In attempting to explain these results, the question naturally arose whether this effect was attained through an increase in the lytic power of the bacteriophage or whether it was due to some effect upon the bacteria. In the series of experiments which follow we have attempted to answer these questions.

II. *The Significance of Bacterial Concentration on the Bacteriolytic Phenomenon Both With and Without Sulfapyridine and Sulfanilamide.*—Staphylococcus phage mixture S1 and single phage BH were tested against four strains in amounts of 0.1 c.c. and 0.35 c.c., and 0.6 c.c. of an eighteen-hour Savita culture, which made suspensions of approximately 50 million, 175 million, and 300 million bacteria per cubic centimeter (Table II). It is evident that with both SP and SN the small bacterial inoculations gave complete clearing with phage with all strains, while the higher bacterial concentrations did not.

TABLE II

CULTURE	0.1 c.c.			0.35 c.c.			0.6 c.c.		
	Sav.	Sav. + SP	Sav. + SN	Sav.	Sav. + SP	Sav. + SN	Sav.	Sav. + SP	Sav. + SN
H									
4 hours	4+	4+	4+	4+	4+	4+	3+	3+	3+
24 hours	4+	4+	4+	4+	4+	4+	4+	4+	4+
6 days	—	4+	4+	—	4+	4+	—	4+	4+
D									
4 hours	4+	4+	4+	4+	4+	3+	2+	±	±
24 hours	4+	4+	4+	4+	4+	4+	4+	4+	4+
6 days	—	4+	4+	—	—	4+	—	—	—
Bu									
4 hours	1+	1+	1+	±	±	±	—	—	—
24 hours	4+	4+	4+	4+	4+	4+	2+	±	—
6 days	—	4+	4+	—	—	—	—	—	—
K									
4 hours	4+	4+	4+	—	2+	2+	1+	—	—
24 hours	4+	4+	4+	4+	4+	4+	2+	2+	4+
6 days	4+	4+	4+	—	4+	4+	—	—	—

4+ = Complete clearing and no growth.

4± to ± = Different degrees of clearing.

— = Normal growth.

III. *The Effect of Sulfapyridine and Sulfanilamide Upon the Titer and Lytic Power of Staphylococcus Bacteriophage.*—

1. *The effect after a single twenty-four-hour passage as compared with five subsequent twenty-four-hour passages:* S1 phage was propagated on nine different strains, and No. 43 phage on three of these nine. After one passage seven of nine showed no appreciable difference between the titers of the Savita broth as compared with Savita plus sulfapyridine. In two, however, there was an appreciable lowering of titer in the presence of the drug. After five passages there was no increase or decrease in the titer or lytic ability of these phages, and no appreciable difference between the Savita phage and those containing SP and SN.

2. *The effect of storage in the refrigerator after a single passage:* Bacteriophages S1 and 43 were propagated at the expense of nine and three strains, respectively, in Savita broth both with and without SP or SN. They were then filtered and placed in the refrigerator. Titrations were carried out immediately, and again after six days, ten days, and one month. The titer was essentially

unchanged throughout this period both with and without SP and SN, except for two phages with SP, which were of definitely lower titer after one passage and remained at this titer throughout.

3. *The effect of SP and SN on the adaptation of staphylococcus bacteriophage to partially or completely resistant staphylococcus strains:* Six strains of staphylococci, four of which were partially resistant and two completely resistant to phage, were used for the repeated propagation of phage in Savita containing sulfapyridine and sulfanilamide. The propagated phages were then applied to the original cultures, and it was found that their lytic power had not increased.

IV. *The Effect of Sulfapyridine and Sulfanilamide on Staphylococci in the Absence of Bacteriophage.*—

1. *The effect on five consecutive twenty-four-hour passages in Savita both with and without sulfapyridine and sulfanilamide:* In this experiment, 0.1 c.e. and 0.01 c.e. of an eighteen-hour culture of eight strains of *Staphylococcus aureus* (three susceptible, two partially susceptible, and three resistant to phage) were inoculated into tubes containing 5.5 c.e. of Savita, Savita plus SP, and Savita plus SN, respectively, and incubated for twenty-four hours. One-tenth cubic centimeter of these cultures was then transplanted into similar media on four successive days. Readings were made after three, six, and twenty-four hours of incubation, and the clear tubes were plated for possible growth. Results of the effect of SP and SN on the tubes inoculated with 0.01 c.e. of these eight cultures and their four subcultures in respective media are presented in Table III. It is seen that all three strains which were completely resistant to phage were immediately inhibited by both SP and SN, and two of these were completely destroyed by SP in the course of the passages. On the other hand, the five strains of staphylococci which were completely or partially susceptible to phage, suffered only a transitory inhibition by SP and were practically not affected by SN.

2. *The effect of one twenty-four-hour passage on varying dilutions of bacteria:* In an attempt to study the action of SP and SN (dilution 1:10,000) on *Staphylococcus aureus* more closely, 0.1 c.e. of dilutions 10^{-2} and 10^{-4} of eighteen-hour cultures were taken beside the dilutions made with 0.1 c.e. and 0.01 c.e. of undiluted culture. In order to ascertain the number of bacteria per cubic centimeter of the suspension and its increase or decrease after contact with the drugs, a loopful (approximately 0.01 c.e.) of the highest dilution was plated immediately, and again after five and twenty-four hours of incubation. Growth in liquid media was followed after five, twenty-four, and forty-eight hours, and the clear tubes containing SP and SN were observed during two weeks of incubation. Fifteen strains of staphylococci, of which five were susceptible to phage, seven partially susceptible, and three entirely resistant, were used. Representative results of these experiments are presented in Table IV. An immediate plating of 0.01 c.e. of all the tubes which had been inoculated with 0.1 c.e. of the 10^{-4} dilution of eighteen-hour cultures of all 15 strains showed two to 34 colonies. After five hours of incubation similar transplants showed that the number of colonies on all plates increased considerably. However, in the presence of SP there were only one-half to one-fourth as many colonies as in plain Savita. After

TABLE III

	CU(SU)			O(SU)			PA(SU)			BU(PS)		
	SAV.	SN	SP	SAV.	SN	SP	SAV.	SN	SP	SAV.	SN	SP
Passage 1 Plating	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0
Passage 2 Plating	++++ 0	++++ 0	++++ 0	++++ 27	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0
Passage 3 Plating	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0
Passage 4 Plating	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0
Passage 5 Plating	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0
	K(PS)			PI(RE)			MN(RE)			KR(RE)		
	SAV.	SN	SP	SAV.	SN	SP	SAV.	SN	SP	SAV.	SN	SP
Passage 1 Plating	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0
Passage 2 Plating	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0
Passage 3 Plating	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0
Passage 4 Plating	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0
Passage 5 Plating	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0	++++ 0

- = No visible growth; no growth on plating.
 ± to + = Various degrees of growth; ± showing hardly visible growth.

++++ = Normal growth.

IC = Innumerable colonies.

203 and other numbers = Number of colonies.

0 = Not plated.

SU = Susceptible.

PS = Partially susceptible.

RE = Resistant.

TABLE IV
EFFECT OF 10⁻⁴ DILUTION OF SULFAPYRIDINE AND SULFANILAMIDE ON VARIOUS AMOUNTS OF STAPHYLOCOCCUS AUREUS IN SAVITA BROTH

	0.1 c.c.				0.1 c.c.				0.1 c.c.			
	UNDILUTED CULTURE				DILUTION 10 ⁻¹				DILUTION 10 ⁻²			
	SAV.	SP.	SN.	SAV.	SAV.	SP.	SN.	SAV.	SAV.	SP.	SN.	SAV.
<i>Culture O Susceptible to Phage</i>												
Original colony count	++++	++++	++++	++++	++++	++++	++++	++++	++++	+	5	5
Growth—5 hours	++++	++++	++++	++++	++++	++++	++++	++++	++++	65	13	3
Colony count—5 hours	++++	++++	++++	++++	++++	++++	++++	++++	++++	+	+	+
Growth—24 hours	++++	++++	++++	++++	++++	++++	++++	++++	++++	70	+	+
Colony count—24 hours	++++	++++	++++	++++	++++	++++	++++	++++	++++	+	+	+
Growth—48 hours	++++	++++	++++	++++	++++	++++	++++	++++	++++	+	+	+
<i>Culture An Partially Susceptible to Phage</i>												
Original colony count	++++	++++	++++	++++	++++	++++	++++	++++	++++	15	9	10
Growth—5 hours	++++	++++	++++	++++	++++	++++	++++	++++	++++	529	179	233
Colony count—5 hours	++++	++++	++++	++++	++++	++++	++++	++++	++++	+	+	+
Growth—24 hours	++++	++++	++++	++++	++++	++++	++++	++++	++++	+	+	+
Colony count—24 hours	++++	++++	++++	++++	++++	++++	++++	++++	++++	+	+	+
Growth—48 hours	++++	++++	++++	++++	++++	++++	++++	++++	++++	+	+	+
<i>Culture Ku Partially Susceptible to Phage</i>												
Original colony count	++++	++++	++++	++++	++++	++++	++++	++++	++++	31	22	34
Growth—5 hours	++++	++++	++++	++++	++++	++++	++++	++++	++++	+	+	+
Colony count—5 hours	++++	++++	++++	++++	++++	++++	++++	++++	++++	1396	267	429
Growth—24 hours	++++	++++	++++	++++	++++	++++	++++	++++	++++	+	+	+
Colony count—24 hours	++++	++++	++++	++++	++++	++++	++++	++++	++++	+	+	+
Growth—48 hours	++++	++++	++++	++++	++++	++++	++++	++++	++++	+	+	+
<i>Culture Ad Resistant to Phage</i>												
Original colony count	++++	++++	++++	++++	++++	++++	++++	++++	++++	29	24	29
Growth—5 hours	++++	++++	++++	++++	++++	++++	++++	++++	++++	+	+	+
Colony count—5 hours	++++	++++	++++	++++	++++	++++	++++	++++	++++	555	146	93
Growth—24 hours	++++	++++	++++	++++	++++	++++	++++	++++	++++	+	+	+
Colony count—24 hours	++++	++++	++++	++++	++++	++++	++++	++++	++++	+	+	+
Growth—48 hours	++++	++++	++++	++++	++++	++++	++++	++++	++++	+	+	+

Colony count was made by spreading upon a blood plate of a loopful (0.01 c.c.) of 5.5 c.c. Savita tube inoculated with 0.1 c.c. of 10⁻² and 10⁻⁴ dilution of eighteen-hour Savita culture.

- = No growth in Savita and on plating.

± to +++ = Various degrees of growth in Savita.

++++ = Normal growth in Savita and on plating.

* = Growth appeared after several days of incubation

IC = Innumerable colonies.

twenty-four hours of incubation the 10^{-4} dilution of each of the 15 cultures grown in SP displayed no visible growth. Upon further incubation it was noted that all but three grew eventually. Of these three destroyed strains, two were resistant to phage and the third was phage susceptible. All three strains when inoculated in 0.01 c.c. amounts of undiluted culture were again the only ones which gave no visible growth in SP tubes.

The effect of SN, although generally lower, was most pronounced in regard to the same three strains.

Out of seven strains used in all of the experiments, which were completely resistant to phage, six proved to be most susceptible to the bacteriostatic and bactericidal effect of SP and SN.

Of all 15 strains susceptible and partially susceptible to phage, only one showed a susceptibility to SP and SN similar to that of phage-resistant strains. Although the others were always affected by SP, they were destroyed by this drug only when they were in high dilution. When the bacteria were not destroyed within twenty-four hours, visible growth always developed upon further incubation.

V. Morphologic Changes Under the Action of Sulfapyridine and Sulfanilamide.—SP and SN not only inhibited the growth of certain strains in broth, but also altered to some extent the appearance of colonies when subcultured on a blood agar plate. Changes in size, shape, pigment production, and hemolysis were observed. Some colonies were degenerated and indented or pin point in size, presenting the appearance similar to changes after contact with bacteriophage. Some were normal yellowish, some were white, others were semi-transparent, and still others were bright yellow. Some colonies were normally hemolytic, others were nonhemolytic, while a few were surrounded by a large zone of hemolysis. One culture treated with SN showed colonies surrounded by a double zone of hemolysis. Microscopically, the cultures grown in SP and SN showed the following morphologic changes: The size of some of the cocci was increased for two to six times. They were not as sharply outlined, the staining was less deep, and some seemed to be encapsulated.

VI. Effect of SP and SN on Phage Susceptibility of Staphylococci.—The phage susceptibility test on six strains of *Staphylococcus aureus*, which was carried out after twenty-four and forty-eight hours' contact of the bacteria with SP and SN at 37° C., showed no change in phage susceptibility of any of the strains. When the susceptibility tests were carried out after five days' contact with SP and SN, the results were inconclusive. Some strains became more resistant and others became more susceptible. Constant results could not be obtained.

B. The Effect of Sulfapyridine (SP) and Sulfanilamide (SN) Upon the E. coli Bacteriophage Phenomenon

I. The Degree and Permanency of Bacteriolysis Both With and Without SP and SN.—The effect of SP and SN upon *E. coli* and *E. coli* phages was studied along the same lines as the experiments with staphylococci. Table V presents results of the susceptibility tests of *E. coli* phage mixture B4 against ten strains of *E. coli* carried out with 0.1 c.c. of an eighteen-hour culture and 0.25 c.c. of

phage in 5.5 c.c. of Savita broth with and without SP and SN (dilution of 1:10,000). The effect of SP and SN upon the bacteria, in the absence of phage, was noted at the same time. From this table one can see that phage in Savita alone cleared seven of ten cultures within four hours, but only three maintained visible clearing for twenty-four hours, and two of these developed growth upon further incubation. The same phage set in Savita, plus SP, produced complete clearing after four hours of the same seven strains, and maintained it for twenty-four hours. Four of these strains maintained lysis for a week in the incubator, while three became cloudy. Two other cultures, which were partially cleared after four hours and remained so after twenty-four hours, later became a little more cloudy in the presence of SP. One of the ten cultures was completely resistant to phage and failed to undergo any lysis even in the presence of phage and SP. Contrary to the results obtained with staphylococci, the addition of SN to *E. coli* phage-bacteria mixtures had much less effect than SP.

TABLE V

LYSIS READING		CULTURES									
		1	2	3	4	5	6	7	8	9	10
Phage B4 in Savita	After 4 hours	4+	2+	4+	2+	4+	4+	4+	~	4+	4+
	After 24 hours	-	3+	4+	2+	-	4+	4+	-	-	-
	After 1 week	-	-	4+	-	-	2+	1+	-	-	-
Phage B4 in Savita + SP	After 4 hours	4+	2+	4+	3+	4+	4+	4+	~	4+	4+
	After 24 hours	4+	4+	4+	3+	4+	4+	4+	~	4+	4+
	After 1 week	-	3+	4+	1+	4+	4+	4+	-	1+	2±
Phage B4 in Savita + SN	After 4 hours	4+	2+	4+	2+	4+	4+	4+	~	4+	4+
	After 24 hours	-	3+	4+	3+	±	4+	4+	-	-	-
	After 1 week	-	-	4+	-	-	4+	-	-	-	-
<i>Controls</i>											
Plain Savita	After 4 hours	-	-	-	-	-	-	-	-	-	-
	After 24 hours	-	-	-	-	-	-	-	-	-	-
Savita + SP	After 4 hours	1+	2+	1+	2+	2+	2+	2+	±	1+	±
	After 24 hours	1+	1+	1+	±	±	1+	1+	-	1+	1+
Savita + SN	After 4 hours	1+	1+	±	1+	1+	1+	-	-	±	-
	After 24 hours	±	±	±	-	-	±	±	-	-	-

4+ = Complete lysis.

3+ to 1+ = Various degrees of lysis.

± = Doubtful.

- = No lysis or normal growth.

Table V also shows that the SP alone produced a marked bacteriostatic effect upon most of the strains. The effect of SN was less than SP both in degree and extent.

The experiment was repeated with 35 more strains of varying resistance to phage, and similar results were noted. It was found that there was a definite correlation between the potency of phage against a given strain and the degree and duration of lysis attained by phage upon addition of SP. The cultures which were not completely cleared by phage in plain Savita within eight hours, showed, in Savita plus SP, a greater degree of lysis, but still it was not complete. The cultures which were completely cleared by phage in plain Savita within eight hours, but by twenty-four hours showed a secondary growth, were maintained clear in SP tubes for a period of twenty-four hours or longer. In other words, the SP prevented or delayed secondary growth.

Furthermore, the cultures that remained clear in Savita for at least twenty-four hours but gave growth upon plating on blood agar, were completely destroyed in the presence of SP. The strains that were permanently destroyed by phage in Savita were, of course, in no way affected by the addition of SP.

II. *The Effect of Sulfapyridine and Sulfanilamide on the Titer, Lytic Ability, and Range of Activity of E. coli Phages.*—Bacteriophage mixture B4 and the individual bacteriophage MK were employed in these experiments and were propagated at the expense of three different strains. All these strains were susceptible to phage B4, but only two of the three were susceptible to phage MK, the third being partially susceptible. The titrations were carried out after one and after five twenty-four-hour passages. At the same time, the first group of phages, incubated for twenty-four hours in the presence of SP, SN, and in plain Savita, were placed in the refrigerator and were titrated after five days, two weeks, one month, and six months. When titrated after twenty-four hours' contact with SP and SN, both B4 and MK phages, propagated on three susceptible strains, suffered no drop of titer, but in the presence of one of these drugs increased tenfold instead of one hundred times as did the control. However, the effect of SP was quite pronounced when bacteriophage MK was propagated at the expense of the partially susceptible strain. In this instance, while the titer of the bacteriophage was lowered to 10^4 in Savita and Savita plus SN, phage developed normally and reached a titer of 10^7 in the presence of SP. This experiment was repeated with the same results, and in general confirmed similar findings obtained with staphylococcus bacteriophage when these were propagated on the partially susceptible strains.

The titration of B4 and MK after five twenty-four-hour passages showed no significant differences in the titer of the phages, whether propagated on any of the three strains in SP, SN, or plain Savita. There was also no significant decrease in the original titer after two weeks, or after one month of contact with the SP and SN in the refrigerator at 4° C. A slight decrease of the titer of phage was occasionally noticed after six months of contact. These results fully agreed with similar tests with staphylococcus bacteriophages.

The lytic ability and the range of activity of *E. coli* phages was usually unaffected by contact with SP, and only when phage was propagated on partially susceptible strains was the phage with SP slightly higher than its Savita control.

Innumerable attempts to increase the lytic ability of *B. coli* phages to partially susceptible strains by adding SP ended in complete failure.

III. *The Effect of Sulfapyridine and Sulfanilamide on E. coli Strains and Their Subsequent Susceptibility to Bacteriophage.*—The same technique was used as with staphylococcus cultures. In no case was there complete destruction of the strain in the course of several passages when the undiluted culture was used and there was no significant difference between the phage-susceptible and phage-resistant strains in their degree of sensitivity to the effect of SP. The effect of SN was even less than that of SP.

On the other hand, when the experiments were carried out with an inoculation of 0.1 c.c. of undiluted bacterial culture and compared with the 10^{-2} and 10^{-4}

dilutions of the bacterial cultures, it was found that SP occasionally destroyed the two higher dilutions, but did not significantly affect the undiluted cultures (see Table VI). The undiluted cultures were in general more susceptible to the bacteriostatic action of SP than the staphylococci, but the higher dilutions were less susceptible to the *bactericidal* action of SP than the staphylococci. The effect of SN again ran parallel with SP but was always less pronounced.

TABLE VI

EFFECT OF 10-4 DILUTION OF SP AND SN ON VARIOUS AMOUNTS OF *B. coli* IN SAVITA BROTH IN THE ABSENCE OF BACTERIOPHAGE

CULTURE	0.1 C.C. OF UNDILUTED CULTURE			0.1 C.C. IN DILUTION 10 ²			0.1 C.C. IN DILUTION 10 ⁴		
	SP+		SN+	SP+		SN+	SP+		SN+
	SAV.	SAV.	SAV.	SAV.	SAV.	SAV.	SAV.	SAV.	SAV.
Culture I (Phage Susceptible)							8	5	6
Immediate plating							200	26	55
4-hour plating							++++	-	++++
24-hour plating							-	-	-
4-hour Savita reading	++	+	+	±	-	-	-	-	-
24-hour Savita reading	++++	+++	+++	++++	-	+	++++	-	++
48-hour Savita reading	++++	++++	++++	+++	-	++++	++++	-	++++
Culture D (Phage Partially Susceptible)							3	7	12
Immediate plating							++	++	++
4-hour plating							++++	++	++++
24-hour plating							-	-	-
4-hour Savita reading	++++	+++	++++	+	±	+	-	-	-
24-hour Savita reading	++++	+++	++++	++++	+++	++++	++++	-	++++
48-hour Savita reading	++++	++++	++++	++++	++++	++++	++++	++	++++
Culture M (Phage Resistant)							13	5	12
Immediate plating							250	135	200
4-hour plating							++++	++++	++++
24-hour plating							-	-	-
4-hour Savita reading	++++	++++	++++	+	±	±	-	-	-
24-hour Savita reading	++++	+++	++++	++++	+++	++++	++++	+	++++
48-hour Savita reading	++++	++++	++++	++++	++++	++++	++++	++++	++++

Colony count was made by spreading approximately 0.01 c.c. of the tube containing 0.1 c.c. of dilution 10⁻⁴ of eighteen-hour culture in 5.5 c.c. of Savita, Savita plus SP, and Savita plus SN upon a quadrant of blood plate.

+++ = Normal growth on plating or in Savita.

- = No growth on plating or Savita.

+++ to ± = Various amounts of growth on plating and Savita, ± being the smallest and +++ the most abundant.

Neither SP nor SN produced any significant morphologic changes in regard to size, form, or other characteristics of the colonies of *E. coli* or significant microscopic morphologic changes in the organisms themselves.

E. coli strains, which were subcultured or maintained up to five days in the incubator in the presence of SP and SN, showed practically no change in their susceptibility to phage.

C. *The Effect of Various Concentrations of Sulfapyridine Upon the Bacteriophage Phenomenon*

In order to ascertain whether concentrations other than 1:10,000 (10 mg. per cent) of SP (which bacteriophage might encounter in the body) exerted a similar effect upon bacteriophage and bacteria, dilutions of 1:1,000, 1:50,000, 1:200,000, and 1:500,000 were studied. The action of these dilutions of SP upon *E. coli* and staphylococcus cultures was tested simultaneously and compared with the dilution 1:10,000. This was carried out with eight strains of each species in the presence and absence of bacteriophage. The experiments showed that SP dilution 1:10,000, in conjunction with bacteriophage, was somewhat more effective than 1:1,000 or 1:5,000 dilutions. The dilutions 1:200,000 and 1:500,000, on the other hand, had absolutely no effect upon the bacteriophage phenomenon.

SUMMARY

We are reporting herewith a study of the effect of sulfapyridine and sulfanilamide upon staphylococcus and *E. coli* cultures and their respective phages with regard to the lytic phenomenon. This study was carried out with three staphylococcus phages against 50 strains of staphylococci and two *E. coli* phages, together with 45 strains of *E. coli*.

1. The addition of sulfapyridine in the dilution 1:10,000 to staphylococcus and *E. coli* phage suspensions with partially susceptible bacterial strains definitely increased the bacteriolysis and often resulted in complete destruction of all bacteria. The best results were attained with bacteriophages capable of maintaining lysis for at least eighteen to twenty-four hours, and with an initial bacterial inoculation not exceeding 50 million bacteria per cubic centimeter.

2. The use of sulfapyridine with weaker phages, which maintained lysis for less than eighteen hours with 50 million bacteria per cubic centimeter without the drugs, usually resulted in prolongation of lysis up to several days, but complete sterilization was seldom attained.

3. Dilution 1:10,000 (10 mg. per cent) of sulfapyridine produced the most favorable effect upon the bacteriophage phenomenon. The effect of dilution 1:1,000 and 1:50,000 was slightly below that of 1:10,000 SP dilution. Dilutions 1:200,000 and 1:500,000, however, did not exert any effect upon the bacteriophage phenomenon.

4. Both sulfapyridine and sulfanilamide exerted a bacteriostatic as well as a bactericidal effect on *Staphylococcus aureus* and were capable of destroying dilute bacterial suspensions, usually not exceeding 5,000 per cubic centimeter. The effect of SP was much greater than that of SN, and this was further shown by its pronounced activity against higher bacterial concentrations. The rapidity, duration, and degree of bacteriostatic and bactericidal effect varied for each strain and was most pronounced and complete against six of seven phage-resistant strains.

5. Both sulfapyridine and sulfanilamide were capable of producing macroscopic morphologic changes with regard to the size, shape, pigmentation, and hemolytic properties of the colonies of staphylococci and also certain microscopic changes on the organisms themselves.

6. Both SP and SN produced some inconsistent changes in the state of phage susceptibility of completely and partially phage-susceptible strains, whereas they had no effect on the state of phage resistance of completely phage-resistant strains.

7. Sulfapyridine possessed a pronounced bacteriostatic and bactericidal effect upon *E. coli*, and was capable of destroying a suspension not exceeding 5,000 bacteria per cubic centimeter of phage-susceptible and partially susceptible strains. When confronted with heavy suspensions of these strains, its bacteriostatic effect was usually more pronounced than on similar suspensions of staphylococci; however, its bactericidal power on *E. coli* was less than on staphylococci.

8. Sulfanilamide caused no significant change in the degree of bacterial lysis of *E. coli* strains with phage, and had no significant bacteriostatic or bactericidal effect on *E. coli* cultures.

9. Sulfapyridine and sulfanilamide added for the first time to staphylococcus and *E. coli* phages propagated on susceptible bacteria occasionally decreased the bacteriophage titer (not more than ten times) and its lytic power. However, upon repeated passages or prolonged contacts the titer and lytic activity of phage usually were re-established to their original level. Preservation of phage in 1:10,000 dilution of SP for six months did not significantly change its original titer. However, the adaptation of phage to partially susceptible strains was more successful in the absence of SP, indicating that SP prevented an increase of potency of phages.

CONCLUSIONS

1. Sulfanilamide and sulfapyridine do not interfere significantly with the lytic action of specific bacteriophage on *Staphylococcus aureus* or *B. coli* in vitro when these organisms are completely susceptible to the corresponding phage.

2. With strains of these organisms, which are not completely destroyed by the lytic action of the corresponding bacteriophage, the associated use of sulfapyridine, and to a less extent sulfanilamide, frequently completes the destruction of the bacteria in vitro—a synergistic effect.

3. Sulfanilamide or sulfapyridine may be used together with specific bacteriophage in the treatment of staphylococcus or *B. coli* infections. There may be a synergistic effect with these combinations in vivo.

4. Bacteriophage is generally more bactericidal on the corresponding staphylococci and *E. coli* than either sulfanilamide or sulfapyridine.

5. In the treatment of staphylococcus and *E. coli* septicemia, the use of potent bacteriophage, when it is available, should not be delayed because of the attempted use of sulfanilamide or sulfapyridine.

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REFERENCES

1. Jern, H. Z., and Meloney, F. L.: Effect on Bacteriophage of Prontylin, Prontosil, Sulfapyridine and Other Antiseptics and Dyes Used in Surgical Practice, *J. LAB. & CLIN. MED.* 24: 1017, 1939.
2. Jern, H. Z., and Meloney, F. L.: Studies in Bacteriophage II. Methods of Maintaining and Testing in the Potency of Bacteriophage, *J. LAB. & CLIN. MED.* 20: 862, 1935.
3. Longacre, A. B., Jern, H. Z., and Meloney, F. L.: The Treatment of Staphylococcus Septicemia With Bacteriophage, *Surg. Gynec. & Obst.* 10: 1, 1940.

VARIATION OF THE ORGANIZED SEDIMENT FOLLOWING PYRIDIUM ADMINISTRATION IN URINARY INFECTIONS*

A TECHNIQUE FOR THE STUDY OF THE ORGANIZED SEDIMENT VOLUME

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IN THE papers by Reynolds, Wilkey, and Choy,⁴ and Morrissey and Spinelli,² the effect of phenylazo-alpha-alpha-diamino-pyridine monohydrochloride on the symptomatology of urinary infections was adequately evaluated. This study is restricted to observations of the effect of pyridium on the variation in the amount of the organized urinary sediment. Although it was undertaken primarily for the purpose of sediment study, observations as to burning, dysuria, and nocturia were also carefully noted in an attempt to correlate the reduction of the urinary sediment with a decrease in the severity of these symptoms.

The terms "organized" and "unorganized" sediment are used as defined by Hawk and Bergeim.¹ In this paper, granular, epithelial, blood, and other pathologic casts will not be a part of the organized sediment. The volume of the organized sediment was selected for study because the total sediment, which may consist to a great extent of the unorganized sediment, bears no direct relationship to the severity of the infection. In a twenty-four-hour urine collection the amount of the unorganized sediment is a function of the diet, the pH, and the external temperature. The amount of unorganized sediment is also a function of the urease enzyme of the bacteria, which acts at various rates, depending upon the amount of urease, the amount of urea, the amount of buffer phosphates, the ionic strength of the solution, the pH, and the temperature. Thus a voluminous precipitate of salts may occur even if only small amounts of pus cells, epithelial cells, and bacteria are present. Since the unorganized sediment precipitate depends upon many factors apart from the infection, it is unsatisfactory material to work with if one is interested in studying the effect of a drug in clearing up infections. Therefore, in this report, the aim is to exclude, as much as possible, the effect of unorganized sediment from the determinations of the organized sediment volume.

While this work was in progress the paper of Morrissey and Spinelli² was published in which tentative studies of sediment variation following pyridium administration were made. No mention was made as to control or technique of their sediment studies. They report "a reduction of one-half to two-thirds of the amount of urinary sediment in operative cases when the drug was used." Although pyridium was given to these patients preoperatively and postopera-

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†Commercially available under the trade name "pyridium."

tively, it is not specified whether the sediment studies were made preoperatively, postoperatively, or both. Since it is stated that these patients were "operative," we assume that postoperative determinations of sediment were made.

Obviously, sediment studies made soon after an operation are comparatively less significant, since the operation is designed to relieve the primary cause from which the infection developed. Thus the condition should clear up spontaneously. Although the drug may do it faster, it is impossible to separate the factor of spontaneous resolution due to better drainage from that of the action of the drug. Only those cases in which infections persist for months after operation were considered suitable for the sediment studies reported here.

In selecting material for this study, the following postulates were established and the choice of patients were so made:

1. Low nonprotein nitrogen. Patients with a high nonprotein nitrogen or those who show extensive kidney damage cannot properly eliminate the educt.*
2. Demonstrable pus cells in a fresh urine specimen—6 to loaded per high-power field.
3. No granular, epithelial, or blood casts, and not more than a trace to 1+ of albumin. These findings, if present, will give false values to the sediment determinations.
4. No gross hematuria, the variation of which from day to day gives sediment values that may have no relation to the severity of the infection.
5. Female patients only can be used:
 - (a) Those who have only a small number of vaginal epithelial cells in the urine during the nonmenstrual period.
 - (b) Those past the menopause.
 - (c) Those who would cooperate in reporting menstrual cycle, so that determinations would not be made during that period.
6. If the patient is a postoperative case, sediment studies should not be started unless the infection persists one or two months after the operation for reasons given in the discussion by Morrissey and Spinelli.³
7. Patients who are being sounded cannot be used for sediment studies, since soundings injure the tissues and increase sediment.

PROCEDURE

The twenty-four-hour urines were collected in brown bottles of from 2 to 4 liter capacity, depending upon the output of the patient. Five cubic centimeters of a 2 per cent alcoholic solution of thymol were added, and the bottle was rotated so that all of its interior was coated by the solution. This is preferable to adding crystals, since the crystals interfere with the reading of the sediment in the calibrated end of the centrifuge bottle. The daily urine volume was measured, so that results could be expressed in both sediment per 100 c.c. and total sediment. A preliminary twenty-four-hour collection without pyridium

*Pyridium elimination product.

was tested for organized sediment by the process described below. For approximately two weeks sediment determinations were run on the subsequent twenty-four-hour periods. This was done in the same manner as that of the preliminary specimen, using pyridium in dosages of 600 to 900 mg. per day.

The twenty-four-hour specimen was well shaken and a portion of about 150 c.c. was placed in a beaker. The pH was adjusted to 5.5 ± 0.2 using 1:1 hydrochloric acid or 1:1 ammonium hydroxide solution. Nitrazine paper was used as the indicator. The pH of the urine was checked with a quinhydrone electrode, and the results agreed very well with the nitrazine paper.

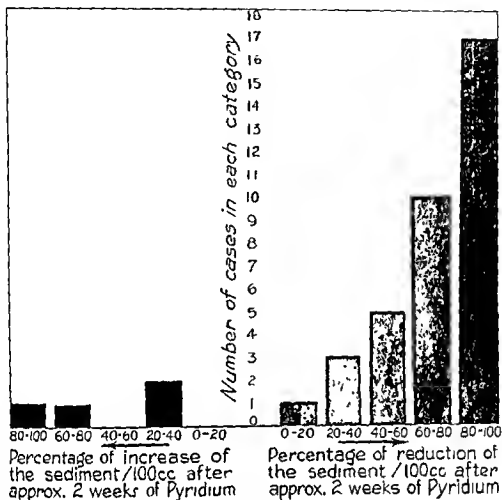
In the case in which the twenty-four-hour urine was at a pH of 5.5 or lower, an excess of ammonium hydroxide was added until a pH of 7.5 to 8.0 was reached, after which the urine was then re-acidified to a pH of 5.5. This was done to dissolve the conjugated pyridium elimination product which sometimes precipitates at these pH values on standing for twenty-four hours and also to dissolve any uric acid which might have been precipitated.

The pH 5.5 was selected because this is near the pH at which urine is voided in normal persons and for the reasons given in the following discussion. At this pH the calcium phosphates, magnesium ammonium phosphates, calcium carbonates, and most of the ammonium urates are dissolved. Thus almost all the salts on the alkaline side which might occupy a very large volume and give false information to the determination of the "organized" sediment will be completely avoided. The only salt that will not dissolve readily at this pH is calcium oxalate, and while it is present in many cases, it contributes a negligible amount to the volume of the precipitate. A pronounced case of oxaluria was not seen during the course of these studies, even though the normal cases were medical students who were under great stress and anxiety due to scholastic examinations.

On the acid side, between pH 5.5 and pH 7, there is a danger of some sodium urate² precipitation, but the patients usually have an output of about 2,000 c.c., and in twenty-four hours, at room temperature, very little of the sodium urate will precipitate unless the patient has a fever.

The determinations of the sediment were run in Goetz phosphorous centrifuge bottles with a volume of 100 c.c. and a tip of 1 c.c. capacity, and calibrated in 0.05 c.c. graduations from which estimations of ± 0.01 c.c. could be made. The specimens adjusted to a pH of 5.5, as indicated above, were centrifuged for fifteen minutes at the rate of $1,800 \pm 300$ r.p.m. At this speed, for the given time, variations in sediment volume are negligible for a few minutes before or after the fifteen minutes' interval, so that it can be concluded that the packing of the sediment has reached a relatively constant point. The nebeccula, which consists of mucin, is insoluble at this pH. It descends in the centrifuge bottle as a light voluminous opalescent flocculation and is not to be considered as a part of the organized sediment. The separation between the nebeccula and the solid opaque organized sediment is usually sharp, so that no difficulty in reading the sediment is encountered. In the case that the separation is not distinct, an error in reading of about ± 0.03 instead of the 0.01 is given, and the best estimation possible is made. This type of difficulty does not occur very often.

It is granted here that in a twenty-four-hour specimen, especially in alkaline solution, cytolysis tends to decrease the sediment volume, but a compensating factor of protein swelling tends to keep this error down. Since the sediment in succeeding twenty-four-hour samples of the same patient is compared, this error will be relatively constant over a short period.



Graph 1.—Distribution of 40 cases of urinary infections with regard to organized urinary sediment variation per 100 c.c. of urine following pyridium therapy for approximately two weeks.

While this work was undertaken principally for the purpose of sediment study, the symptoms of burning and nocturia were carefully noted. In almost all cases in which these symptoms were reported, there was a marked decrease in nocturia and in the severity of the burning following the administration of pyridium. Some cases were spectacular with respect to the rate at which a severe burning would disappear, giving evidence of the reported analgesic power of pyridium. In some of these cases a severe burning would be completely gone after twenty-four to forty-eight hours' use of pyridium. Results such as these are a confirmation of all the clinical studies made by previous investigators. This part of the work was done in an effort to correlate the reduction and disappearance of the symptoms with a decrease in the sediment.

In Table I no attempt was made to classify the types of cystitis. Not all patients were cystoscoped. The diagnoses of those patients who were not cystoscoped were made on the basis of symptomatology and demonstrable pus cells in a fresh urine specimen. Undoubtedly there were a few cases of pyelocystitis in this group. The symptoms of burning, dysuria, and nocturia are listed opposite the percentage of reduction to facilitate comparison of the symptomatology changes with the reduction in sedimentation per 100 c.c. Where

TABLE I*

CYSTITIS	PRELIM- INARY SED./100 C.C.	SED./100 C.C. WITH PYRIDIUM 2 WEEKS	AMOUNT OF REDUCTION SED./100 C.C.	% OF REDUCTION SED./100 C.C.	BURNING OR DYSURIA	NOCTURIA
1 M	0.78	0.13	0.65	83.3	--	---
2 M	0.55	0.17	0.38	69.1	--	---
3 M	0.65	0.1	0.55	84.6	--	---
4 M	0.30	0.03	0.27	90.0	--	---
5 M	0.24	0.07	0.17	70.8	--	---
6 M	0.43	0.6	0.17 inc.	39.2 inc.	--	---
7 M	1.30	0.1	1.20	92.3	--	---
8 M	2.3	0.15	2.15	93.5	--	---
9 F	0.20	0.25	0.05 inc.	25.0 inc.	--	---
10 M	0.15	0.30	0.15 inc.	100.0 inc.	--	---
11 M	0.63	0.15	0.48	76.2	--	---
12 M	0.30	0.15	0.15	50.0	Less	Less
13 M	0.25	0.24	0.01	4.0	Less	Less
14 M	4.0	1.1	2.9	72.5	--	---
15 M	2.0	0.18	1.82	91.0	--	---
16 M	1.8	0.43	1.37	76.1	Less	Less
17 M	2.0	0.45	1.55	77.5	Less	From 20x to 2x
18 M	0.20	0.35	0.15 inc.	75.0 inc.	--	---
19 M	0.57	0.05	0.52	91.2	Gone	From 5x to 1x
20 M	1.0	0.20	0.80	80.0	Less	From 3x to 1x
21 F	0.32	0.03	0.29	90.6	Gone	From 4x to 1x
22 M	0.45	0.26	0.19	48.0	Less	From 10x to 4x
23 M	0.34	0.25	0.09	26.5	Less	Same
24 M	0.45	0.21	0.24	53.3	--	---
25 M	0.25	0.03	0.22	88.0	Gone	Same
26 F	0.25	0.20	0.05	20.0	Gone	---
27 M	0.48	0.24	0.24	50.0	Gone	None
28 M	0.34	0.09	0.25	73.6	--	---
29 M	0.15	0.02	0.13	86.7	Less	Less
30 M	0.70	0.10	0.60	85.7	Gone	From 15x to 8x
31 M	0.25	0.13	0.12	48.0	Same	Same
32 M	0.70	0.18	0.52	74.3	Less	Less
33 M	0.12	0.03	0.09	75.0	Less	From 15x to 8x
34 M	0.14	0.02	0.12	85.7	None	None
35 M	0.65	0.02	0.63	97.0	Less	None
36 M	0.65	0.45	0.20	30.4	--	---
PYELONEPHRITIS						
1 M	1.1	0.13	0.97	88.2	--	---
2 F	0.40	0.06	0.34	85.0	Less	Less
3 M	0.26	0.06	0.20	77.0	Less	Less
RENAL INFECTION FOLLOWING NEPHROLITHOTOMY						
1 M	2.00	0.30	1.70	85.0	Less	Less
NORMAL CASES						
1 M	None	None	0	---	No symptomatology	
2 M	0.02	None	0.02	---		
3 M	None	None	0	---		
4 M	None	None	0	---		
5 M	None	None	0	---		

*The percentage of reduction of the total sediment would give a result similar to Table I. Because of errors in volume, inherent in the collection of twenty-four-hour specimens from hospital and ambulant patients, The author feels that the expression of the results in sedimentation per 100 c.c. is more justified and better controlled than the total sediment.

M = male.

F = female.

inc. = increase.

there is a line through the space or spaces in the symptomatology columns, no information was available. *Gone* means that symptoms were present and have completely disappeared. *Less* means symptoms were present and the severity has decreased considerably. *Same* means that symptoms were present and that they have not improved. *None* means that symptoms were not present. In no case was the severity of the symptoms increased following pyridium administration. Other symptoms recorded were not put on the chart due to space limitations. Wherever the expression "sed. per 100 c.c." occurs, it will be understood to mean cubic centimeters of sediment per 100 c.c. of urine.

COMMENTS

Table I and Graph 1 show strikingly the effect of pyridium on the reduction in the amount of organized sediment in cases of urinary infections. Eighty per cent of these cases show a decrease in sediment of 50 per cent or more. In those cases in which observation of burning and dysuria were recorded, 95 per cent showed a decrease in the severity or a disappearance of these symptoms. As can be seen from the chart, a good correlation exists between the reduction of the sediment and a decrease in the severity of the symptoms. In a few cases an improvement of the symptomatology took place without a corresponding decrease in the sediment. Normal persons do not have any appreciable organized sediment before or after pyridium.

SUMMARY

1. A technique has been presented for the determination of the organized urinary sediment volume.
2. Normal persons have a very low organized sediment before and after pyridium administration, between 0.0 and 0.02 c.c. per 100 c.c.
3. Pyridium is very effective in reducing the organized sediment in cases of urinary infection, especially cystitis and pyelonephritis.
4. With very few exceptions the reduction of the organized sediment is accompanied by a decrease or disappearance of the subjective symptoms.

REFERENCES

1. Hawk, P. B., and Bergeim, O.: *Practical Physiological Chemistry*, ed. 10, Philadelphia, 1931, P. Blakiston's Son & Co.
2. Maslow, L. A.: A Study of the Hydrogen Ion Concentration and Crystallography of Normal Urine, *J. Urol.* 35: 674, 1936.
3. Morrissey, J. H., and Spinelli, A. N.: An Experimental Study of the Anesthetic and Analgesic Properties of Pyridium, *J. Urol.* 44: 381, 1940.
4. Reynolds, J. S., Wilkey, J. L., and Choy, J. K. L.: Clinical Application and Results of Pyridium Therapy, *Illinois M. J.* 78: 544, 1940.

THE MODE OF ACTION OF BRAN*

I. EFFECT OF BRAN UPON COMPOSITION OF STOOLS

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INTRODUCTION

THE extensive use of bran as a laxative and in diets justifies an inquiry into its various properties. This report deals mainly with the physical and chemical qualities of bran stools. It is aimed to discover the mode of action of bran and to elucidate, as far as possible, the effect the alimentary tract has upon bran and the effect bran has upon the alimentary tract.

METHOD OF STUDY

These investigations were carried out on approximately 200 healthy persons, for the most part male medical students between 20 and 30 years of age. A few healthy women subjects were also used.

To make the studies of practical value these persons were placed on uncontrolled and unrestricted diets, just as are the public consumers of bran. The individuals collected their stools in specially prepared asphalt paper bags† for three to six days (prebran period). This control period was followed by a second period, which varied in different experiments from three to fourteen days, during which time one ounce of bran (Kellogg's "All-Bran") was added to the daily diet (bran period). A third period of several days followed (after-bran period). All persons had normal bowel habits; stools were obtained regularly every day with very few exceptions.

At present the only consistent criterion of the laxative action of bran is the change in stool weight. Whenever this has been definitely increased, we speak of laxative action. When the weight is decreased or not changed, it is spoken of as lack of such action. It should be admitted that this criterion is not the only one. Bulk is perhaps more important, but it has not been possible to measure it accurately. The consistency of the stool may be still more important for it may possibly be more constantly affected by bran, and "penetrometric" studies to investigate this matter are reported. The frequency of the stools is important, but it was not possible to secure a record that gave significant results in these normal persons of fixed habits. The rapidity with which the bran stool marked with barium passes through the alimentary tract has been carefully

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studied,¹ but it does not seem to give better results than those based upon the weight, and a comparison of the results using weight as the criterion with results using speed as the criterion will be discussed in a subsequent report.

Bran contains approximately 8 per cent of crude fiber. Thus the usual one ounce serving of bran supplies only 2.4 Gm. of this material. This crude fiber comprises about 2 per cent of the total dry stool weight. Obviously, the bulk-forming properties of bran are not due to this small quantity of crude fiber per se.

The hydrophilic properties of bran crude fiber are negligible. Therefore, water absorption by bran crude fiber is likewise eliminated as an important factor in the formation of bulky stools.

A change in stool consistency almost always appears after the ingestion of bran: bran stools are generally softer. The change in consistency could not be accounted for by changes in moisture content, since this varied little from the control stools.

To determine moisture, about 2 Gm. of well-mixed stool were weighed on a watch glass and dried in an oven at 100° C., cooled, and weighed again; the drying and weighing were continued until the weight was constant.

In one series 7 persons (18.4 per cent) showed no change in the moisture content of bran stools. Eight persons (21 per cent) showed a decrease in moisture content, the decrease being slight (average 4.5 per cent, maximum 6 per cent). Twenty-three persons (60.6 per cent) showed an increase in moisture to a very slight degree (average 4.9 per cent, maximum 17 per cent). Bran stools definitely appeared soft, but it is impossible to distinguish between 5 per cent increase or decrease in water content by the appearance alone. While moisture may play a part in the increased softness of bran stools, the increase is not sufficiently constant to account for any substantial change in consistency. The softness is, no doubt, also due to gas. To determine whether softness is a constant change in all bran stools and to express the change quantitatively, penetrometric studies were undertaken.

PENETROMETER STUDY

The penetrometer is intended to measure the consistency of homogeneous materials. Stools are not homogeneous and, in addition, the quantity of gas which is present before expulsion of the stool from the gastrointestinal tract is no longer present. Gas is lost in transferring the specimen from the original container to the measuring apparatus and during the testing. Thus far the results of the penetrometric studies have been entirely unsatisfactory.

SPECIFIC GRAVITY OF STOOLS

Attempts to measure accurately the gas content of stools have thus far proved unsatisfactory. A considerable portion of gas is lost at defecation and during transportation, storage, and the handling of stool specimens.

Bran stools, probably due to gas content, have a lower specific gravity than control stools. It is, therefore, probable that the increase in bulk is actually greater than the increase in weight, in some cases to a considerable extent.

However, an attempt was made to develop a method for measuring the specific gravity of stools. None of the methods recommended in the literature proved satisfactory for the purpose. A liquid was required that would not dissolve or penetrate into the stool; therefore, water and similar fluids were excluded. After searching for a nonaqueous liquid and one that would not evaporate too rapidly, a petrolatum-benzin (10 per cent benzin) mixture was chosen. Approximately 5 Gm. of feces were placed in a weighed graduated centrifuge tube carefully weighed again and the petrolatum-benzin mixture was added from a burette up to the 15 c.c. mark. The weight of the specimen divided by the difference between 15 and the number of cubic centimeters of liquid added gives the specific gravity of stool.

A comparison of weight and volume in the bran and afterbran periods showed that in some cases the difference in bulk is much more obvious than that in weight. This method was abandoned because of difficulties encountered in its use.

CHEMICAL EXAMINATIONS

The pH of the stools, the total fat content, the amount of volatile fatty acids, and the relative proportion of butyric to acetic acid were determined.

Olmsted² stated that the laxative effect of bran is produced in large part by the volatile fatty acids originating from the breakdown of bran by bacteria.

Hydrogen-ion determinations (pH) were made using a quinhydrone electrode potentiometer. Solid stools were diluted two to three times with saline. In one series, the stools of 36 persons (65.4 per cent) showed a slight decrease in hydrogen-ion concentration, and those of 19 (34.6 per cent) showed a slight increase during the bran period.

The stool fat was determined by adding a quantity of 1 per cent alcoholic hydrochloric acid solution equivalent to five times the weight of the sample. This mixture was stirred well and dried on a water bath. The product obtained was extracted in a Soxhlet for twelve hours with ether. This procedure gives the total amount of nonvolatile fatty acids. During the bran period 18 per cent of 67 persons showed an increase in stool fat content, and 82 per cent showed a decrease.

Kjeldahl nitrogen determinations were made on a known quantity of dried stool. The nitrogen content of the stools was examined in the prebran, bran, and afterbran periods of some of the individuals who were on controlled diets. An increase in nitrogen was found during the bran period, probably due to a more rapid passage of the stools through the gut. The increase in nitrogen was not very marked and soon returned to normal in the afterbran period.

The volatile fatty acids in the stools were determined by releasing them from their salts by the addition of a stronger acid. About 150 c.c. of 10 per cent oxalic acid and water were added to approximately 10 Gm. of fresh stool (accurately measured) in a flask which was connected to a condenser system. The acids which passed over with the steam were taken up in a known quantity of N/5 sodium hydroxide. After the distillation was finished, the solution was titrated with N/5 sulfuric acid. The difference between the amount of alkali

in the receiving flask before distillation and the amount after distillation as indicated by the titration with N/5 sulfuric acid, gives the quantity of volatile fatty acids.

These volatile fatty acids are found to be composed chiefly of acetic and butyric acids. However, there is no correlation between an increase in stool weight and the relative proportion of acetic to butyric acid.

The amount of volatile fatty acids varied greatly in all the experiments. No uniform relationship between butyric and acetic acid was noted. Volatile fatty acids are present in the gut combined with cations, such as potassium and calcium. It must be considered doubtful whether the salts of these acids contribute to the laxative action of bran. A very careful statistical study of the question showed that the correlation between volatile fatty acids and laxative action (as measured by increased stool weight) is so slight (0.09) that it cannot be considered significant from the statistical standpoint.

CONCLUSIONS

1. The most significant change in stools following the addition of bran to an uncontrolled diet is a softening and an increase in stool weight.
2. Bran stools show an increase in bulk greater than the increase in weight. There is little variation in the percentage of water.
3. There is an increase in volatile fatty acids, chiefly acetic and butyric acids, but this increase is not proportional to the increase in weight.
4. The proportion of acetic to butyric acid showed no consistent correlation with laxative action as measured by an increase in stool weight.

We are grateful to Miss Elizabeth M. Adles for assistance in statistical studies. Protocols for all experiments are available.

REFERENCES

1. Fantus, B., Kopstein, G., and Schmidt, H.: Roentgen Ray Study of Intestinal Motility as Influenced by Bran, *J. A. M. A.* 114: 404, 1940.
2. Olmsted, W. H., Curtis, G., and Timm, O. K.: Cause of Laxative Effect of Feeding Bran Pentosan and Cellulose to Man, *Proc. Soc. Exper. Biol. & Med.* 32: 141, 1934.

LABORATORY METHODS

GENERAL

THE PRODUCTION OF ARTIFICIAL TUMORS IN THE BRAINS OF CATS*

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THE method used in these experiments for the production of so-called artificial tumors in the brains of animals is not a new one. Rather, it is a modification of the method employed by van Schultén,¹ Hill,² and later in 1909 by Cushing and Bordley.³ Van Schultén screwed small tubes into the skulls of animals for the purpose of injecting substances into the subarachnoid space and for recording cerebrospinal fluid pressures. Hill's study of acute cerebral compression was based in part on a similar technique. His historical review of the subject is especially interesting. Cushing and Bordley used the same method in their studies, demonstrating the mechanical relationship that exists between increasing intracranial pressure and retinal edema. In some of their experiments paraffin was injected, and they noted no permanent "effect beyond that occasioned by immediate local destruction of tissue." A search of the literature has revealed no further application of this method as we have used it.

These experiments demonstrate a method whereby a foreign body, which can be increased gradually in size over a period of weeks or months, may be introduced into the brain of an unanesthetized animal and controlled in its growth. Further, they indicate some of the effects of such foreign bodies. Van Schultén's tube has been modified to resemble a grease cup, and the cups have been filled with sterile beeswax softened to a proper consistency with lipiodol. The mixture was injected slowly by turning the cup down a little each day, and has been found of itself to be relatively inert for the periods of time employed (Fig. 3). Only slight inflammation was observed in the walls around the wax. For each experiment, under aseptic conditions, a grease cup plug was screwed into a trephined hole in the skull of an animal. In all 34 cats were used. The dura under the hole was cut away before the plug was implanted and afterwards the skin was closed around it. Tubes were placed in the skulls over most of the accessible parts of the cerebral hemispheres. Records were made of the general health of the animals, and the placing reactions, described by Bard and Brooks,⁴ were tested at about daily intervals, both before and after the cups

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were turned down. Observations on the optic disks, and x-ray pictures of the skulls of some of the animals were made at longer intervals. Meningitis and brain abscesses developed in several of the animals and these were discarded. With the appearance of pressure symptoms, the cats were anesthetized with nembutal, and the vascular system was injected with 10 per cent formalin. The brains were then removed for gross, and in some instances microscopic, examination.

RESULTS

It was found that the shape of the tumors could be controlled within limits by varying the relative concentration of wax and lipiodol. Mushroom-shaped masses lying under the dura were produced by the use of a mixture predominantly lipiodol (ratio 5:1). Long cylindrical masses were formed when the mixture was chiefly beeswax (Fig. 1). Spherical tumors lying below the surface of the brain were formed when a mixture of two parts of lipiodol and one of wax was used (Figs. 2 and 4).

Histologic examination of the tumors and surrounding brain tissue in sections stained with hematoxylin and eosin showed that the foreign body produced its direct effect by compression, displacement, and distortion of the normal architecture of the brain, and indirectly in some instances by blockage of the cerebrospinal fluid passages so that hydrocephalus developed. A complete fibrous capsule containing capillaries, and continuous with the dura was found around most of the tumors (Fig. 3). The pia-arachnoid appeared to be fused with this capsule at the surface of the brain where the greatest proliferation of fibrous tissue was present. A small number of leucocytes was found in the capsule, particularly on its inner surface, and some fibrin. No changes in the character or number of the supporting cells of the brain tissue itself were observed with the hematoxylin and eosin stains.

A modified Weigert's stain was applied to sections obtained from the brain of Cat 5. The rounded tumor (Fig. 2) was located just back of the motor area under the lateral gyrus on the right; it had penetrated 2 cm. downward into the brain, pushing the corpus callosum downward about 8 mm. Sections through the brain about the foreign body showed, as might be expected, a marked decreased staining capacity of the myelin sheaths of the fibers in the corpus callosum immediately under the mass. Thirty-seven days elapsed during the production of the mass, and approximately 1 c.c. of the wax mixture was injected.

In these experiments the first effect of the increasing size of the foreign body was a disturbance of the normal placing reactions. This was true no matter where the grease cups were located. However, different placing reactions in different legs were primarily affected, depending on the location of the masses. The order of appearance of deficiencies in the placing reactions resulting from wax tumors placed on different areas appears to offer some evidence as to the localization of these reactions in the cortex. On the other hand, for proper interpretation, it must be considered that transmitted pressure can be an important factor in the production of these deficiencies.

The accumulation of a mass in the middle portion of the anterior sigmoid gyrus, a region known to be a contralateral foreleg area primarily, produced first a deficiency in the abduction response in the contralateral foreleg. In another animal, pressure of the foreign body on the posterior sigmoid gyrus led to a deficiency in the contact response in the contralateral forelegs and hind-legs first. This evidence of localization is to be expected, since this region, which has been shown to be the cortical area of motor representation for these extremities (Ward and Clark⁵), has also been shown by strychninization (Dusser de Barenne⁶) to be a part of the area for sensory representation of the contralateral foreleg and to a less extent, the contralateral hindleg.

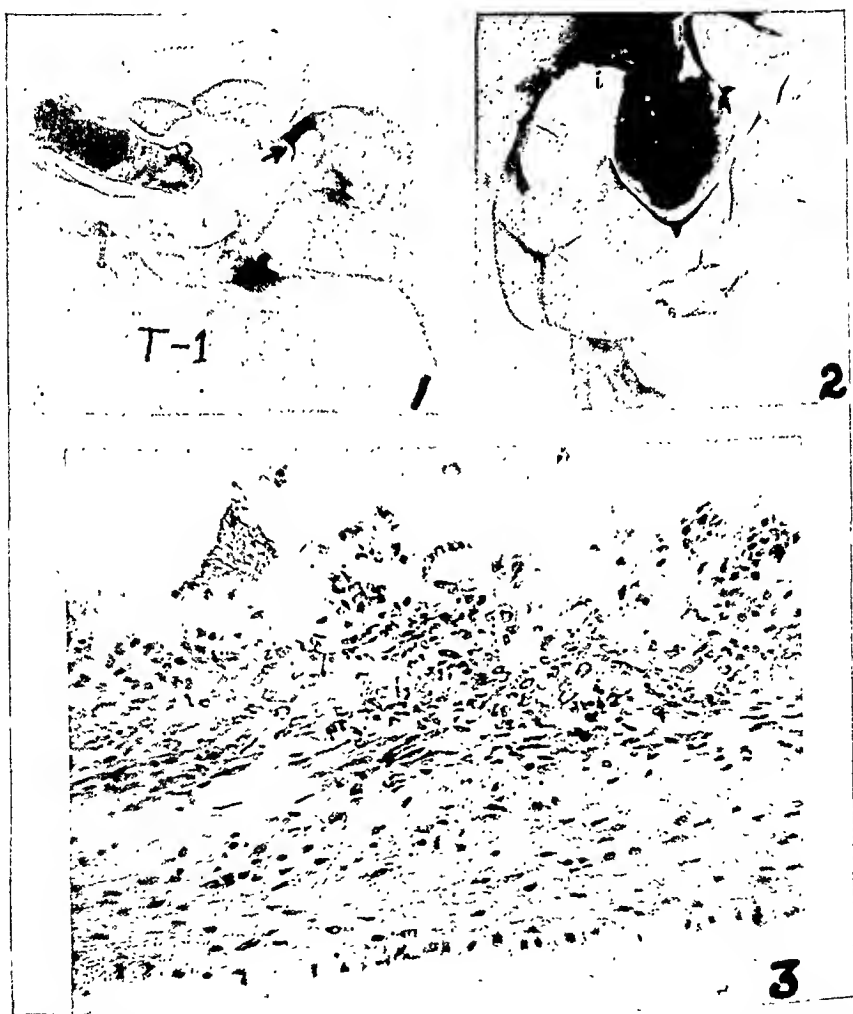


Fig. 1.—Cylindrical foreign body formed over a period of sixty-nine days (cup turned down 14 times, one turn each time). Marked hydrocephalus developed, and herniation of the cerebrum (tentorial surface) under the bony tentorium is seen at arrow.

Fig. 2.—Spherical foreign body formed over period of thirty-five days; 20 (turns) increments of wax were added. A degeneration of the myelin sheaths was noted with Weigert's stain in the corpus callosum.

Fig. 3.—Section through wall around the foreign body and the corpus callosum from cat's brain pictured in Fig. 2. Ependymal lining of lateral ventricle seen below. (See text. H. & E. Oil $\times 215$.)

In contrast to the relatively immediate deficiencies that occurred when the foreign bodies were placed in the anterior one-third of the brain, an increasing delay in the appearance of symptoms was found when the masses were more and more posteriorly placed; that is, more increments of wax (from 3 to 10 turns of the grease cup) were required before the appearance of placing reaction deficiency.

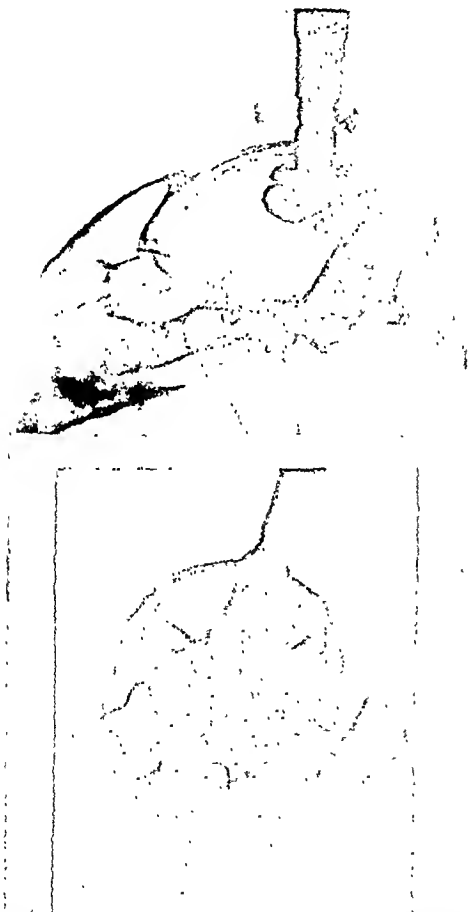


Fig. 4.—X-ray pictures of spherical foreign body formed in nineteen days (15 turns).

In 8 cats with masses on the posterior two-thirds of the cerebrum, the first alteration of the normal activity was noted to be a slowing in the contact reaction in the contralateral hindleg. Bard and Brooks have shown the integrity of the placing reactions to be dependent on the area immediately around the cruciate sulcus and the most anterior end of the lateral gyrus. The results of experiments in which the masses were located in various parts of the posterior two-thirds of the cerebrum demonstrate the effect of the pressure exerted either on a "center," or on nerve pathways leading to or from such a "center." It does not appear likely that the cortical "centers" subserving the placing reactions are those affected by the pressure of these remote masses, because when pressure is exerted directly against these regions, either different placing reactions or combinations of placing reactions are affected first. A selective effect of pressure transmitted downward to the centrifugal or centripetal cortical pathways appears to offer a more likely explanation. Whether this is due to a selective loss of function like that which occurs with pressure on a peripheral nerve (Gasser and Erlanger⁷), or whether it is the result of pressure on the blood vessels supplying these regions is not known.

Remissions of symptoms frequently occurred when more than twenty-four hours elapsed between successive additions of wax. This is illustrated in an animal (Cat 19) with the grease eup on about the middle of the right lateral gyrus close to the midline. The eup was turned down one turn each day for the first seven days. The contact placing reactions after the first forty-eight hours became progressively slower and smaller in both hindlegs. The responses of the forelegs remained normal. No increment was added on the eighth day, and within the next twenty-four hours all reactions became normal. The contact response in the hindlegs again was slightly reduced on the eleventh day after the additions of wax were resumed on the ninth and tenth days. X-ray pictures of the skull showed that the mass occupied a position on both sides of the midline in the posterior one-third of the cerebral cranial cavity (Fig. 4).

The location of the masses in the cranial cavity appeared definitely related to the time of appearance and intensity of the symptoms of compression. The slowly enlarging foreign bodies produced displacement of the brain tissue, and in some instances this displacement was enhanced by the additional factor of hydrocephalus, the hydrocephalus being caused by the indirect blockage of the drainage passages of the cerebrospinal fluid by the foreign bodies. Autopsy revealed the presence of pressure cones in the animals that survived for several weeks or more, though dilated ventricles were present only in some of these. In those with undilated ventricles the tumors were frequently large and usually situated so as to produce most of their displacement from the front of the cranial cavity. When situated more posteriorly, tumors formed by slower and less frequently added increments of wax tended not to be associated with dilated ventricles, even though tumors similarly situated did lead to distended ventricles if they were formed more rapidly. In all probability the factor of rate of growth accounts for the lack of early symptoms in certain human cases in which enormous, slow-growing tumors have eventually manifest themselves.

As the wax masses were increased in size, the effects on the animals presented a fairly constant pattern. A blurring of the edges of the optic disks with engorgement of the veins was seen. The cats sat very quietly, back humped, nose down, neck extended, feet bunched. When they were handled, they were found to be tense and at times cried angrily and lashed their tails. They were usually unsteady on their feet, and the placing and hopping reactions were lost in all legs. They appeared to prefer to lie on the side opposite the mass if the mass was definitely asymmetrical in its position. The pupils were usually dilated and responded to light slightly, or not at all; and vision appeared to be impaired in that the animals often ran into objects as they moved about.

Terminally the animals exhibited some of the symptoms characteristic of decerebrate rigidity. They were no longer able to stand, and their heads and necks were thrown back. While the cats were lying undisturbed, their forelegs were extended and retracted, and their hindlegs were partially flexed. The Stutz reaction was easily elicited in the forelegs. Local reflex activity was unimpaired. Slow running movements occurred at times in the course of this stage. Death followed shortly. For example, in Cat 34 the mass was located close to the midline and in the middle third of the cerebrum and had been produced rapidly (16 turns, 2 each day). Death occurred on the eighth day, though no symptoms appeared until the seventh day. Autopsy showed slightly dilated ventricles, a cerebral herniation at the tentorium and a cerebellar pressure cone at the foramen magnum. A bony tentorium cerebelli in the cat is probably responsible for some of the symptoms of decerebration that are to be observed in these acute stages. Compression of the brain stem under the tentorium occurs with the herniation of the pulvinar and the tentorial surface of the cerebrum. This herniation was observed in all animals allowed to go to this advanced stage (Fig. 1).

SUMMARY AND CONCLUSIONS

The experiments demonstrate a method whereby foreign bodies that simulate growing benign tumors can be placed on various parts of the brains of animals.

The masses give rise to localizing symptoms, and a chronic state with evidence of increased intracranial pressure can be produced.

An acute state of increased intracranial pressure may develop with rapid injection of the wax mixture; hydrocephalus may develop and become a factor in the production of the symptoms. Some symptoms of decerebration characterize this state.

The masses can be visualized in the living animal by x-ray pictures.

The experiments indicate in another way that parts of the motor area in the cat subserve certain placing reactions in different extremities. Of these placing reactions it was found that the contact reaction was the most labile in that pressure exerted on parts of the cerebrum other than the motor region caused an elimination of this reaction before abolishing the others.

REFERENCES

1. Van Schultén, M. W.: Untersuchungen über den Hirndruck, mit besonderer Rücksicht auf seine Einwirkung auf die Circulationverhältnisse des Auges, Arch. f. klin. Chir. 32: 947, 1885.

2. Hill, Leonard: *The Physiology and Pathology of the Cerebral Circulation*, An experimental Research, London, 1896, J. & A. Churchill.
3. Cushing, Harvey, and Bordley, James: *Observations on Experimentally Induced Choked Disc*, Bull. Johns Hopkins Hosp. 20: 95, 1909.
4. Bard, P., and Brooks, C. M.: *Localized Control of Some Postural Reactions in the Cat and Rat Together With Evidence That Small Cortical Remnants May Function Normally*, A. Research Nerv. & Ment. Dis. Proc. 13: 107, 1932.
5. Ward, James W., and Clark, Sam L.: *Specific Responses Elicitable From Sub-divisions of the Motor Cortex of the Cerebrum of the Cat*, J. Comp. Neurol. 63: 49, 1935.
6. Dusser de Barenne, J. G.: *Experimental Researches on Sensory Localization in the Cerebral Cortex*, Quart. J. Exper. Physiol. 9: 355, 1916.
7. Gasser, H. S., and Erlanger, J.: *The Role of Fiber Size in the Establishment of a Nerve Block by Pressure or Cocaine*, Am. J. Physiol. 88: 581, 1929.

A METHOD FOR PREPARING SMEARS AND SECTIONS OF ASPIRATED STERNAL MARROW*

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THE diagnostic value of biopsies of sternal marrow is so firmly established that the time may soon arrive when failure to examine the marrow of a patient with an obscure hematologic disorder will have medicolegal significance. Many instruments have been devised, and several methods have been suggested to facilitate the procedure of sternal biopsy. These fall into two general categories: aspiration and trepanation techniques. The latter possesses a number of advantages as pointed out by Custer,¹⁻³ Dameshek, Henstell, and Valentine,⁴ and Hynes.⁵ It also has important limitations, as emphasized by Young and Osgood.⁶ Some workers have devised instruments by means of which solid fragments of spongy bone may be removed from the sternum by a modified trephine technique.^{7, 8} However, all the various trepanation methods have the serious disadvantage of being relatively formidable procedures which cannot be repeated at short intervals. The advantages of the aspiration technique are so many that it is the method of choice of most clinicians. It has one serious drawback—the lack of organized tissue makes impossible a well-oriented study of tissue relationships. In order to overcome this difficulty, it has been suggested⁹⁻¹¹ that smears may be made of some of the marrow and the remainder be allowed to clot. The clot may then be fixed, embedded in celloidin or paraffin, sectioned and stained. Reich,¹² and Vogel, Erf, and Rosenthal¹³ concentrated the marrow by centrifuging it and made smears from the buffy coat. The method described below is a further modification of the foregoing and permits the making of smears from sternal marrow both before and after concentration by centrifuging, as well as the preparation of tissue sections. It requires no special instruments, avoids excessive hemodilution, and yields material which is adequate for diagnosis in almost every instance.

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METHOD

An 18 gauge spinal puncture needle is filed through $1\frac{1}{2}$ inches from its shaft, its point is rebeveled, and a fine mark, 0.6 cm. from the tip, is made on it with a file. The purpose of this mark is to provide a convenient guide whereby the operator may judge the depth of penetration of the needle when in use. The usual thickness of the outer table of the sternum is 0.4 cm.; hence, when the file mark is flush with the skin, the point of the needle is usually within the marrow cavity. The puncture site, preferably in the midline between the second and third ribs, is cleansed with iodine and alcohol and infiltrated with novocain solution. The novocain should be injected into the skin, subcutaneous tissues, and periosteum. The puncture needle, with stilet in place, is pushed through the soft tissues and into the outer table of the sternum. The operator should hold the needle in such a way that the file mark is grasped firmly between his thumb and index finger, while the broad base of the needle fits snugly into the palm of his hand. This enables him to push the needle into the marrow cavity without losing control of the instrument.

Penetration of the cortex of the bone is facilitated by rotating the needle slowly as it is pushed in with a steady pressure. When the file mark is flush with the skin, the stilet is withdrawn, and a 5 or 10 c.c. syringe is fitted to the needle. Trial aspiration is made by applying negative pressure to the syringe. Usually marrow mixed with blood is obtained at the first trial. Sometimes it is necessary to remove the syringe, replace the stilet, and go a little deeper in order to obtain marrow. This happens especially if the patient is obese, or if the skin is edematous. Experience soon permits the operator to gauge the thickness of the subcutaneous panniculus while he is infiltrating the tissues with novocain. If, in his judgment, the soft tissues are unusually abundant, he may hold his thumb and index finger "stop" a corresponding distance above the file mark, which is based upon the average thickness of the soft tissues and the outer table of the sternum. The sternal marrow should be withdrawn rapidly, using strong negative pressure. This occasions a moderate feeling of discomfort but aids in dislodging minute plugs of marrow. It is not advisable to withdraw more than 1.0 c.c. of marrow in order to minimize hemodilution. As soon as the syringe is filled to the 1.0 c.c. mark, the needle and syringe should be withdrawn, and their contents emptied into a small test tube containing sufficient dry oxalate to prevent clotting. Pressure is then applied to the sternal puncture site and a sterile dressing is affixed.

Smears are made immediately from the "oxalated" marrow, dried in air, and stained with Wright's stain. The undiluted stain is allowed to act for two minutes, care being taken to avoid evaporation and precipitation of the stain. If some of the stain becomes precipitated, an additional drop or two will dissolve the precipitate. The stain is diluted with distilled water and allowed to remain on the slide for six to eight minutes longer. The stain is washed off with distilled water and the smears may be examined immediately, using the low-power objective to ascertain whether or not they are satisfactory for diagnostic purposes. If the smears are considered satisfactory, the remainder of the marrow is centrifuged at low speed for fifteen to twenty minutes. The

clear supernatant fluid is pipetted off, and additional smears are made from the concentrated buffy layer. These are stained with Wright's stain as previously. The remainder of the marrow is removed gently from the tube and placed on a small piece of dry filter paper, to which it will adhere firmly. The filter paper with adherent marrow is placed in 10 per cent aqueous solution of formalin, dehydrated in alcohol, cleared in xylol, embedded in paraffin, and sectioned. The sections should be from 4 to 6 microns thick, and several duplicate sections



Fig. 1.—A fragment of aspirated sternal marrow. Hematoxylin and eosin stain. Magnification 190 diameters (approximately). The marrow was removed from a white male, aged 22 years. Although the marrow was hypoplastic (confirmed at necropsy), two small fragments of organized marrow tissue were obtained. Both showed a marked granulocytopenia, a moderate decrease of erythropoiesis, and a diffuse fatty replacement of the marrow.

may be mounted on each of two slides. They may be stained with hematoxylin and eosin or with methylene blue, eosin-phloxin.¹⁴ Small fragments of marrow will usually be present in the sections. These frequently appear as well-organized pieces of tissue, even in a marrow which is hypoplastic (Fig. 1). In leucemic marrow, groups of atypical cells often are collected in nests about small vessels (Fig. 2). Occasionally no organized tissue is found in the sections, and sometimes the centrifuged material disintegrates as it is sectioned. However, during the three-year period the method has been in use, very few such failures have been encountered. Moreover, in the occasional case in which no tissue frag-

ments are found, the smears still are available for examination. One other point is worthy of emphasis: in no instance has sternal puncture caused local implantation metastases in my experience and no instance of such a sequel can be found in the available literature. This is of considerable practical importance and is very remarkable in view of the fact that thousands of sternal punctures have been performed during the past few years. It is all the more significant when it is considered that many of these punctures revealed the presence of a variety of malignant neoplasms in the sternal marrow.

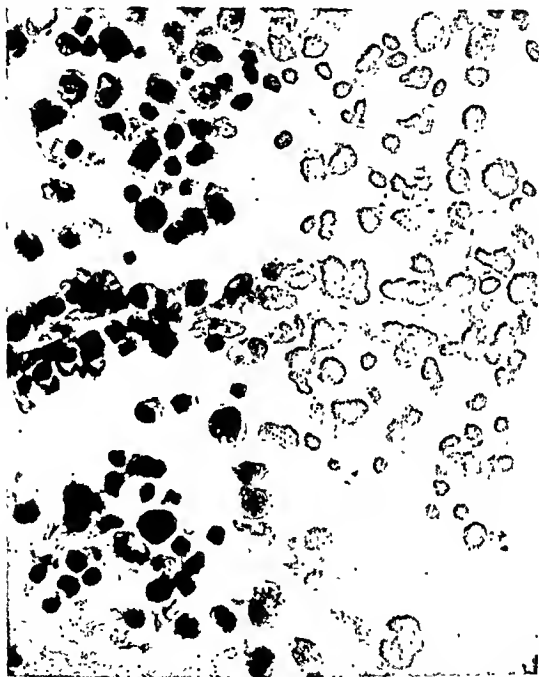


Fig. 2.—Sternal marrow obtained by aspiration. Hematoxylin and eosin stain. Magnification 190 diameters (approximately). The marrow was aspirated from a patient with diffuse plasma cell myelosis. Note the small vessel in the center and the groups of plasma cells in the adjacent perivascular spaces.

SUMMARY

A method is described whereby sternal marrow may be obtained by aspiration and prepared in the form of both smears and tissue sections.

Attention is directed to the fact that no examples of implantation metastases have been encountered following aspiration of sternal marrow.

REFERENCES

1. Custer, R. P.: Studies on the Structure and Function of Bone Marrow; Variability of Hemopoietic Pattern and Consideration of Method for Examination, *J. LAB. & CLIN. MED.* 17: 951, 1932.

2. Custer, R. P., and Ahlfeldt, F. E.: Studies on Structure and Function of Bone Marrow; Variations in Cellularity in Various Bones With Advancing Years of Life and Their Relative Response to Stimuli, *Ibid.* 17: 960, 1932.
3. Custer, R. P.: Studies on the Structure and Function of Bone Marrow. III. Bone Marrow Biopsy, *Am. J. M. Sc.* 185: 617, 1933; Studies on the Structure and Function of Bone Marrow. IV. Bone Marrow in Agranulocytosis, *Ibid.* 189: 507, 1935.
4. Dameshek, W., Henstell, H. H., and Valentine, E. H.: The Comparative Value and the Limitations of the Trephine and Puncture Methods for Biopsy of the Sternal Bone Marrow, *Ann. Int. Med.* 11: 801, 1937.
5. Hynes, M.: Sternal Puncture, *Lancet* 1: 1373, 1939.
6. Young, R. H., and Osgood, E. E.: Sternal Marrow Aspirated During Life. Cytology in Health and in Disease, *Arch. Int. Med.* 55: 186, 1935.
7. Tuohy, E. L., and Gillespie, M. G.: A Trephine Modified to Secure Bone Marrow (Sternal) Biopsies, *J. A. M. A.* 104: 1404, 1935.
8. Favorite, G. O.: An Instrument for Obtaining Bone Marrow, *J. LAB. & CLIN. MED.* 25: 199, 1939.
9. Amprino, R., and Penati, F.: Die Probeexcision aus dem Knochenmark des Brustbeins, *Klin. Wchnschr.* 14: 131, 1935.
10. Limarzi, L. R.: The Diagnostic Value of Sternal Marrow Aspirations, *Illinois M. J.* 75: 38, 1939.
11. Vogel, P., and Bassen, F. A.: Sternal Marrow of Children in Normal and Pathologic States, *Am. J. Dis. Child.* 57: 245, 1939.
12. Reich, C.: A Study of the Diagnostic Value of Sternal Puncture in Clinical Hematology, *Am. J. M. Sc.* 189: 515, 1935.
13. Vogel, P., Erf, L. A., and Rosenthal, N.: Hematological Observations on Bone Marrow Obtained by Sternal Puncture, *Am. J. Clin. Path.* 7: 436, 498, 1937.
14. Gordon, H.: A Methylene Blue Stain for Sections of Formalin-Fixed Marrow, *J. LAB. & CLIN. MED.* 24: 405, 1939.

ISOPROPYL ALCOHOL AS A DEHYDRATING AGENT AND PRESERVATIVE FOR BIOLOGICAL SPECIMENS*

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DURING recent years isopropyl alcohol has been coming into wider use as an antiseptic and sterilizing agent, but little emphasis has been placed on its applicability to general biological routine processes in the laboratory. Its use in the latter connection was first pointed out by Griffin.¹ Isopropyl alcohol, C_3H_7OH , is an inexpensive by-product of the oil industry, with properties very similar to ethyl alcohol. It costs less than tax-free 95 per cent ethyl alcohol. It is not palatable as a beverage and is not subject to the extensive bonds and responsibilities required in the use of tax-free ethyl alcohol. It is a water clear liquid, completely miscible with distilled water. It is sold commercially as anhydrous isopropyl alcohol, with less than 1 per cent of water and other impurities, and as such acts as well as absolute ethyl alcohol in dehydrating procedures. In the preparation of histologic and pathologic material, it is the equal of ethyl alcohol as a dehydrating agent, preservative, and solvent for stains. We have used it extensively in the staining procedure for the diagnosis of parasitic protozoa in fecal smears with excellent results. In fact, we have entirely replaced it for ethyl alcohol for all purposes in our laboratory.

REFERENCE

1. Griffin, L. E.: Practicable Substitutes for Grain Alcohol, *Science* 55: 262, 1922.

*From the Biological Research Institute, Zoological Society of San Diego.
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A PRACTICAL METHOD FOR INTRASTATE EVALUATION OF THE PERFORMANCE OF SEROLOGIC TESTS FOR SYPHILIS*

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INTRODUCTION

THE improvement of laboratory tests used as aids in the diagnosis of disease is a matter of fundamental importance, not only to the medical profession but also to the public as well. Refinements in technique and new discoveries have added much in accuracy in recent years to many laboratory tests; however, constant vigilance must be exercised to insure that new procedures do not sacrifice too much in specificity in the effort to attain speed and sensitivity.

In those states where the State Department of Health maintains a Bureau of Laboratories, consisting of a central laboratory and several branch laboratories, and in those states where private clinical laboratories are officially authorized to perform certain kinds of examinations, under supervision of the State Board of Health, some means of checking performance is essential. This is true particularly where laws are in effect requiring certification of freedom from venereal disease, based on physical examination and certain laboratory tests, before marriage licenses may be obtained. Usually these tests consist of serologic tests for syphilis, and, where indicated, microscopic examinations of smears for evidence of gonorrhea. It is true, of course, that while the results of these laboratory tests per se are not sufficient grounds for denial of a license, the actual tests performed should, nevertheless, be of the highest degree of reliability, i.e., they should be above question as to their sensitivity and specificity.

A survey of the literature relating to the serodiagnosis of syphilis is likely to leave one confused due to the multiplicity of tests from which to choose. It should be remembered, however, that in the last analysis the value of any technique depends, first, on its specificity, that is, its failure to give positive reactions in conditions other than syphilis; and second, on its sensitivity, that is, the proportion of actually syphilitic patients detected by the test. The very multiplicity of available procedures, and the conflicting claims made for them, is sufficient indication that the perfect test has not yet been devised. The most practical way of evaluating this or that particular technique is, therefore, by properly controlled comparisons on parallel series of specimens.

SEROLOGIC CONFERENCES

Within recent years several official attempts have been made to evaluate the merits of various complement fixation and flocculation procedures. In the first place conferences were conducted by the health organization of the League of Nations in 1923, and 1928, and 1930, at which duplicate samples of some hun-

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dreds of sera were tested by the serologists who had devised diagnostic tests for syphilis, each serologist performing his own test.

Subsequently, the first attempt in the United States at determining the relative values of various techniques was made in 1934-1935, under the joint auspices of the United States Public Health Service and the American Society of Clinical Pathologists. Still later, another evaluation study was carried out in 1937, and others followed in 1938, 1939, and 1940, the scope of participation having been broadened to include not only laboratories of the originators of tests but also most state laboratories and some municipal laboratories as well.

As a result of these comparative studies it has been shown that there are at least five serodiagnostic tests for syphilis which are satisfactory in both sensitivity and specificity as long as the technique of the originator is strictly adhered to. However, the studies also showed that marked variations were obtained by laboratories using supposedly the same procedure when the directions for performance given by the originator of a test were not strictly followed.

As an outgrowth of these evaluation studies, critical reviews of procedures have been stimulated, with the result that substandard or obsolete techniques have been abandoned and emphasis has been placed on the importance of strict adherence to all the minor technical details of a described procedure. The net result has been a marked improvement in the sensitivity and specificity of the serodiagnostic tests for syphilis routinely carried out in the participating laboratories.

THE PROBLEM IN ALABAMA

For many years the Bureau of Laboratories of the Alabama State Board of Health has attempted to attain uniformity of procedure in its central and branch laboratories by various means, including the use of check specimens. Efforts have also been made along this line in cooperation with hospital and private clinical laboratories within the state.

Check specimens for the evaluation of serologic tests for syphilis have been somewhat of a problem, however, since completely authenticated specimens from known syphilitic and nonsyphilitic persons, in numbers necessary for adequate evaluation, such as have been furnished in the interstate evaluations of the United States Public Health Service and the intrastate evaluations conducted in Maryland¹ in 1939-1940, and in Georgia² in 1940, have not been available. As a substitute for such specimens, and as a means of making possible continuous weekly checks of serodiagnostic tests for syphilis, the following procedure has, therefore, been used for some time in Alabama.

METHOD

The primary assumption of this method of evaluation is that certain serodiagnostic tests for syphilis have already been demonstrated to be satisfactory by the United States Public Health Service. Consequently, evaluation of the ability of a procedure to detect diminishing amounts of reagin in diluted specimens constitutes an adequate check on the sensitivity of the test in the laboratory being checked. Sufficient negative sera are included over a period of time to evaluate specificity.

Blood specimens received in the central laboratory for serologic test for syphilis are first tested routinely by means of the presumptive Kahn test. Sera that give a negative result by this test, which are to be used in the preparation of check specimens, are set aside and later retested by the presumptive Kahn test, using the serum undiluted and in a 1:5 saline dilution, to assure against technical errors and prozones. Sera reacting negatively to these two tests are pooled for use in preparing check specimens. Diagnostic sera giving any degree of reaction in the presumptive Kahn test are subjected to the standard Kahn test; sera giving a strongly positive reaction with the standard Kahn test are also pooled for use in preparing check specimens. The pooled negative sera and the pooled positive sera are Seitz-filtered separately, to free them from bacteria and undesirable precipitates; such filtration has caused no difference in reactivity so far as parallel specimens have indicated.

Eight dilutions of positive in negative serum—1:2, 1:4, 1:5, 1:8, 1:10, 1:16, 1:20, and 1:30—are prepared in sterile flasks. These dilutions are most easily prepared by making basic dilutions of 1:2 and 1:5, and preparing the higher dilutions from these. These dilutions are transferred to sterile, corked tubes, serially numbered in groups of 10; the numbers on the tubes purposely bear no relationship to the dilution of serum; the ninth and tenth tube in each group are for the inclusion of one tube of undiluted positive serum and one tube of undiluted negative serum. Approximately 200 c.c. of negative serum and 50 c.c. of positive serum have been found ample for preparing the 10 check specimens for 12 to 15 laboratories. Sets of tubes are assembled, and a sheet of instructions as to the inactivation and the method of reporting results is included, and the specimens are mailed to the various laboratories simultaneously. When the results are received, they are tabulated as shown in Table I; errors are checked and a copy of the composite results is sent to each serologist.

INTERPRETATION OF REPORTED RESULTS

From Tables I and II it appears that any laboratory which obtained a 2+ or stronger reaction in its diagnostic test with these particular sera in a 1:5 dilution was running a satisfactorily sensitive test, and for that reason a line is drawn across the tables after the 1:5 dilution. Any laboratory which failed to get a positive reaction in this dilution was considered as conducting an insensitive test. If any error is made in the choice of this critical dilution, it is believed that it is on the side of conservatism, since it is thought that an effort to obtain maximum sensitivity may result in a certain number of false positives, i.e., some loss in specificity.

In Table I it appears that laboratories 1 and 2 underread or overread the sera diluted 1:16 and 1:20, and that laboratories 2 and 6 performed supersensitive presumptive Kahn tests. It also appears that laboratory 7 underread the 1:4 or overread the 1:5 dilutions of its standard Kahn test, whereas laboratory 9 performed an undersensitive presumptive Kahn test.

In Table II, on the particular day these specimens were examined, laboratories 13, 14, and 15 conducted undersensitive complement fixation tests, and laboratory 13 an undersensitive standard Kahn test. In laboratory 12

underreading in the lower dilution or overreading in the higher dilution seems to have occurred in the 1:8 or the 1:10 dilution in the presumptive Kahn test, and in the 1:16 or the 1:20 dilution in the standard Kahn test. The same error seems to have been made in laboratory 14 in the 1:4 and 1:5 dilutions in the Wassermann test. In laboratory 10 a certain degree of false positive reaction was obtained in the negative control serum with an Eagle flocculation test.

SUMMARY

A plan is presented for the conduct of serologic check testing which is being used with success by the Bureau of Laboratories of the Alabama State Department of Public Health. The plan has several important advantages. It is inexpensive. It utilizes materials available in the laboratory every day. It enables checking of performance at weekly intervals throughout the year. By including enough frankly negative sera with those that contain varying amounts of reagin, it checks on both specificity and sensitivity. It readily indicates errors in technique, and it encourages revisions in procedure.

CONCLUSIONS

1. Intrastate evaluations of the performance of serologic tests for syphilis are of great value, not only to the participating laboratories but also to the physicians and to the public.

2. To be of greatest usefulness the evaluation of performance of serodiagnostic tests for syphilis must be made at frequent intervals.

3. A practical method for conducting intrastate evaluations of serologic tests for syphilis, based on the "horizontal method of analysis," has been described.

4. The method described is economical and checks both specificity and sensitivity.

5. On the basis of check testing by the method described, provision is made to assist any laboratory in improving its performance of serodiagnostic tests for syphilis through inspection, and the instruction of serologists in the Bureau of Laboratories of the State Department of Health.

REFERENCES

1. The Maryland Syphilis Serology Survey of 1939-1940, Baltimore Health News 17: 53, 1940.
2. Intrastate Evaluation Study of the Performance of Serologic Tests for Syphilis in Georgia, 1939, Ven. Dis. Inform. 21: 99, 1940.

A SIMPLE MODIFICATION OF WELTMANN'S COAGULATION REACTION*

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NUMEROUS investigators abroad, and Kraemer,¹ Levinson, Klein, and Rosenblum^{2, 3} in this country, have established the value of Weltmann's serum coagulation reaction for diagnosis and prognosis. However, the original technique is too cumbersome to be used as routine procedure. It involves the frequent preparation of ten solutions of calcium chloride, a set-up of at least ten test tubes, and a water bath. The modification outlined here requires only one test tube, one standard solution of calcium chloride, and 0.1 ml. of serum.

TABLE I

	TUBE I	II	III	IV	V	VI	VII	VIII	IX	X
Weltmann's method										
CaCl ₂ /1,000	1.0	0.9	0.8	0.7	0.6	0.5	0.4	0.3	0.2	0.1
mg. CaCl ₂ /5 ml.	5.0	4.5	4.0	3.5	3.0	2.5	2.0	1.5	1.0	0.5
Modification										
ml. of 1% CaCl ₂	0.5	0.45	0.4	0.35	0.3	0.25	0.2	0.15	0.1	0.05
			Exudative zone			Normal zone			Fibrotic zone	
			Shift to left						Shift to right	
			Short band						Long band	

Principle.—A 1:50 dilution of normal human serum with distilled water does not coagulate on heating unless a bivalent electrolyte, such as calcium chloride, is added to make a concentration of 0.4 to 0.5 per thousand. Sera from conditions where exudation prevails, such as pneumonia, acute rheumatic fever, and exudative tuberculosis, require an increased concentration of electrolyte to one per thousand and more. On the other hand, sera from cases where fibrosis and liver damage are prominent, such as fibrotic tuberculosis, subacute atrophy of the liver, cirrhosis of the liver, and septic conditions, coagulate with very low concentrations (0.1 to 0.3 per thousand).

Weltmann placed into ten test tubes 4.9 ml. of solutions, ranging from one per thousand to 0.1 per thousand of crystalline calcium chloride. The integral amounts to 0.1 per thousand or 0.5 mg. calcium chloride per 5 ml. This amount is contained in 0.05 ml. of a 1 per cent solution of calcium chloride, which is the standard solution used in the modification.

Technique.—Into a medium-sized test tube (about 6 by 0.5 inches) place 4.9 ml. of distilled water and 0.1 ml. of clear nonhemolytic serum. Add from a 0.1 ml. pipette 0.05 ml. of 1 per cent calcium chloride (equal to 0.5 mg.). Shake and bring to a boil over a small flame. Cool and repeat the procedure until flocculation has taken place. Note the milligrams necessary to produce floccula-

*From the Arthritis Clinic and Department of Laboratories, White Memorial Hospital, College of Medical Evangelists, Los Angeles.

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tion. Table I gives the conversion into the corresponding calcium chloride concentration and also for comparative purposes the number of the tube of Weltmann's original reaction. Violent boiling should be avoided because it may lead to foaming and loss of fluid. The observation for flocculation should be made after cooling. In practice it is not necessary to proceed by single units of 0.05 ml.

The first boiling can be done after the addition of 0.15 ml. of calcium chloride. This is still lower than the concentration necessary for the flocculation of normal serum and is characteristic of fibrotic processes. If the serum is from known exudative cases, such as pneumonia or active tuberculosis, the test can be started by the addition of 0.25 ml., the upper normal range. After one has acquired enough experience, he can judge by the amount of turbidity after the first boiling approximately how many more units of calcium chloride will still be required, thus shortening the test.

A slight increase in dilution of serum occurs by the addition of calcium chloride to the 1:50 dilution. For the whole range of the Weltmann test the error is not greater than 1 to 10 per cent, which is compensated by the loss of fluid through boiling. A comparison between the original Weltmann test and the modification in 61 cases showed an average difference of less than one tube. In severe exudative cases, such as pneumonia, where flocculation may occur far beyond the range of 1:1,000 (Weltmann's tube I) 15 to 20 tubes would have to be set up. By the modification the severest stages are conveniently and quantitatively determined by the simple addition of the number of units of the calcium chloride necessary for complete flocculation. The results of the test in 307 cases are summarized in Table II.

TABLE II

CONDITION	NUMBER OF CASES	MAXIMUM	MINIMUM (ML. OF 1% CaCl_2)	AVERAGE
Pneumonia	31	1.0	0.3	0.59
Bronchitis, influenza	6	0.45	0.2	0.35
Pulmonary tuberculosis	29	0.65	0.15	0.36
Tonsillitis	14	0.45	0.2	0.26
Inflammation of gall bladder, urinary bladder, sinuses, prostate	34	0.55	0.15	0.3
Fibroid tumors	5	0.5	0.2	0.35
Carcinoma	7	0.5	0.15	0.26
Pregnancy, post partum	32	0.45	0.15	0.25
Arteriosclerosis, hypertension, chronic myocarditis and circulatory disorders	23	0.7	0.2	0.25
Appendicitis	16	0.65	0.15	0.28
Chronic gonorrhea	5	0.55	0.2	0.32
Syphilis	9	0.4	0.2	0.28
Goiter	7	0.3	0.15	0.27
Gout	6	0.35	0.3	0.33
Infectious, rheumatoid arthritis	48	0.55	0.15	0.36
Osteo-arthritis	35	0.45	0.15	0.27

The cases were not selected as to stage or complications. The maximum values indicate the degree of exudation which is brought about primarily either by the disease, such as pneumonia or active tuberculosis, or by the secondary tissue destruction and inflammation, such as occur in carcinoma. The minimum

values are characteristic for subsidence of exudation, development of fibrosis, or a combination of both processes in the chronic stages.

Of the modifications which have been published abroad, Havas' micromethod retains the cumbersome original technique. The only advantage is reduction of the necessary reagents and serum to one-tenth of that required by the Weltmann method. Tenfel's modification uses the same principle as described in this paper, but employs only 0.5 per cent calcium chloride. The error of dilution is, therefore, doubled. To deliver the calcium chloride he uses special droppers which are expensive, not easily available, and introduce another error because they do not deliver the exact unit of calcium chloride that is required.

I wish to express my appreciation to Dr. Orlin B. Pratt, director of the laboratories, Department of Pathology, White Memorial Hospital, College of Medical Evangelists, for providing the facilities of the laboratory for this work.

REFERENCES

1. Kraemer, M.: The Weltmann Test in Diseases of the Liver, *Am. J. Digest. Dis. & Nutrition* 2: 14, 1935.
2. Levinson, S. A., Klein, R. I., and Rosenblum, P.: The Weltmann Sero-Coagulation Reaction. Preliminary Report, *J. LAB. & CLIN. MED.* 23: 53, 1937.
3. Levinson, S. A., and Klein, R. I.: The Value of the Weltmann Serum Coagulation Reaction as a Laboratory Diagnostic Aid; Comparison With the Sedimentation Rate, *Ann. Int. Med.* 12: 1948, 1939.
4. Havas, A.: Weltmann Reaction Micro-Method, *Wien. klin. Wchnschr.* 49: 710, 1936.
5. Teuf, R.: Weltmann Reaction Simplified Technique, *Med. Klin.* 33: 237, 1937.

NITRIC AND SULFURIC ACIDS. A COLORLESS CLEANING MIXTURE FOR GLASSWARE

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MANY scientific workers are apparently unaware of the useful properties of a mixture of nitric and sulfuric acids for cleaning glassware.

The most commonly used cleaning mixture consists of concentrated sulfuric acid containing dissolved sodium or potassium dichromate (or better, chromium trioxide). When freshly prepared, this makes an excellent cleaning solution, but it rapidly becomes spent with use, turning green owing to the reduction of the chromium trioxide by organic materials on the glassware. More of the chromium compound may then be added, but the mixture soon becomes so thick and pasty that it must be discarded. This mixture has the further disadvantage of having a very dark color, so that it is extremely difficult to find small objects and to remove them from the bath. Furthermore, it is claimed¹ that traces of chromium which adhere to the glass can only be removed by very extensive washing, and, therefore, may contaminate biological materials which subsequently come in contact with the glass. This is especially true in the case of fritted glass filters. Traces of chromium have a strongly toxic action on many microorganisms.²

A mixture of concentrated sulfuric and nitric acids has none of these disadvantages. A cold mixture containing about 10 per cent of nitric acid by vol-

ume is very effective in removing traces of grease from glassware (such as pipettes or microscope slides) after a few hours' immersion. When hot, the mixture is extremely effective in oxidizing large amounts of organic material (such as resins, oils, or dried biological fluids) adhering to the glass. In so doing, it often takes on a brown color due to partly oxidized organic matter and liberated nitric oxide, but upon continued heating to complete oxidation, the color of the bath will return to light yellow or light yellow green. Too much organic matter will consume the nitric acid, in which case more can be added as needed. It should be stirred in well, preferably after the mixture has cooled.

If much cleaning is done a convenient method of use is to maintain a bath of sulfuric acid in a large evaporating dish or other acid-resisting container on a gas range or electric hot plate under a hood, and to add a small amount of nitric acid with thorough stirring at the beginning of each day's work, before turning on the heat. A duriron dish is extremely desirable, and avoids the danger of breakage which is always present with porcelain or glass containers. The nitric acid should be well stirred into the cold sulfuric acid, otherwise it will form a layer on the surface and will evaporate rapidly as the bath becomes hot. Such a cleaning bath may be maintained for several months, and need only be discarded when the sulfuric acid has become viscid by the slow accumulation of inorganic salts from the oxidized materials.

The mixture has the disadvantage of fuming somewhat when exposed to the air, especially when heated; hence to avoid breathing the irritating fumes it should be used in a hood with a good current of air. As with the dichromate-sulfuric acid mixture, great care should be taken to avoid splashing the mixture on the hands or clothing, since severe burns may result. Steel crucible tongs are useful in removing glassware from the bath, and such glassware should be well cooled under the hood before rinsing with water. If exposed to the air for several days without heating, the mixture will absorb atmospheric moisture and increase in volume, but the absorbed water rapidly evaporates when the bath is heated again.

The use of chlorates or perchlorates in sulfuric acid cleaning baths has sometimes been proposed, but such mixtures are *extremely dangerous* on account of the explosion hazard and should always be avoided. Sodium or potassium nitrates have also been used, but they have no advantages over nitric acid. The addition of any solid salt will make the bath viscid and shorten its useful life.

Care should, of course, be taken not to heat the mixture so hot that white fumes of sulfuric acid arise, since this will cause a large loss of the acid. If enough nitric acid is present, very gentle heating is usually sufficient to oxidize most organic materials on the glass.

REFERENCES

1. Laug, Edwin P.: Retention of Dichromate by Glassware After Exposure to Potassium Dichromate Cleaning Solution, *Indust. & Engin. Chem., Anal. Ed.* 6: 111, 1934.
2. Richards, Oscar W.: Killing Organisms With Chromium as From Incompletely Washed Dichromate-Sulfuric Acid Cleaned Glassware, *Physiol. Zool.* 9: 246, 1935.

FREEZING OF TISSUES WITH "DRY ICE" FOR MICROTOME SECTIONING OF THE ENTIRE BRAIN*

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IN RECENT YEARS, solid carbon dioxide ("dry ice") has been used in freezing tissues preparatory to sectioning and has been found to have many advantages over the gas. Lindsay and co-workers,¹ and Dunn² described "dry ice" attachments for freezing small blocks of tissue, which can be used with the standard freezing microtome. Mason³ devised a method of using the rocking microtome for sections frozen with carbon dioxide gas.

We have constructed a "dry ice" attachment by which coronal sections of the entire cerebrum may be easily frozen and cut on the larger sledge type celloidin microtome (Minot-MacCallum). For those laboratories which possess a microtome that can accommodate sections 4 by 4 inches (Minot), a smaller attachment was constructed. With this attachment, a coronal section of one cerebral hemisphere, the basal ganglia, whole animal brains, infant brains, kidneys, spleens, etc., may be sectioned.

FREEZING APPARATUS

The freezing attachment consists essentially of (1) a metal plate upon which the slab of tissue is frozen, and (2) a means of keeping the "ice" which in turn chills the tissue, at the same time anchoring the tissue to the plate. Freezing will not occur unless the "ice" is in contact with the plate. After the "ice" is removed, the hardening process continues from the cold plate. With a thin plate, the freezing process is rapid; a thicker plate slows the freezing process but retains the cold longer.

The freezing plate of the larger apparatus consists of sheet aluminum, $\frac{1}{8}$ inch thick and measuring $6\frac{1}{2}$ by 5 inches. This is screwed to a bakelite box,† measuring $6\frac{1}{16}$ by $5\frac{5}{16}$ by $2\frac{5}{16}$ inches, of which it becomes the roof. The end of the box is cut away, and a wooden drawer is built to fit snugly into the box through this end. The drawer (Fig. 1, *D*) contains a slab of "dry ice" (*I*) which is forced against the aluminum plate (*P*) by a false bottom (*F*) mounted on spiral springs (*S*). The springs slide on thin metal tubes (*T*), each of which surrounds a bolt (*B*) which is fastened to the floor of the drawer by a nut (*N*). At the free end of the bolt, a washer (*K*) and nut (*M*) limit the upper excursion of the false bottom. The washer (*W*) helps keep the spring in place. The bakelite box (*X*) is screwed to the object clamp supporting base of the microtome.

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†Molded Bakelite Meter Box, No. K10531, sold by the Lafayette Radio Company, 100 6th Avenue, New York City.

To anchor the tissue more firmly to the freezing plate, moistened filter paper may be interposed, or deep grooves may be cut into the plate.

The smaller apparatus consists simply of an electrical conduit box, 4 by 4 by $1\frac{5}{8}$ inches, and a false bottom of hard rubber mounted on springs, as in the

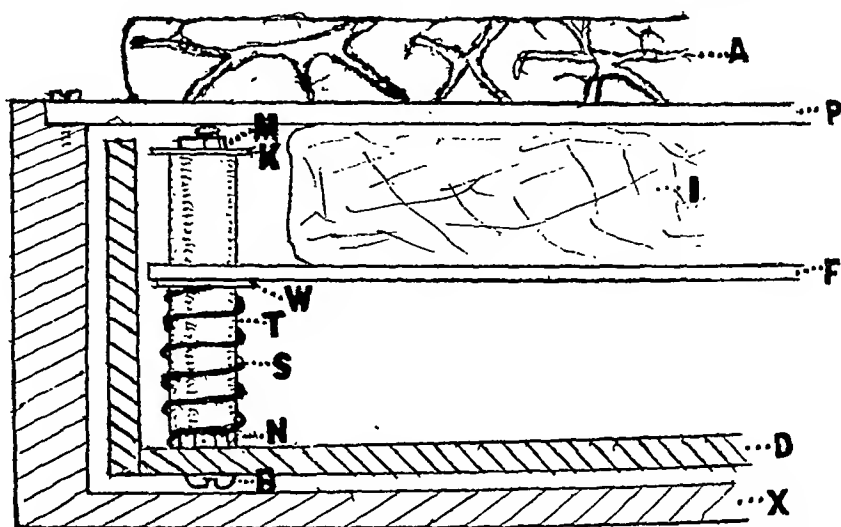


Fig. 1.—Diagram of freezing attachment. See text for explanation of letters.

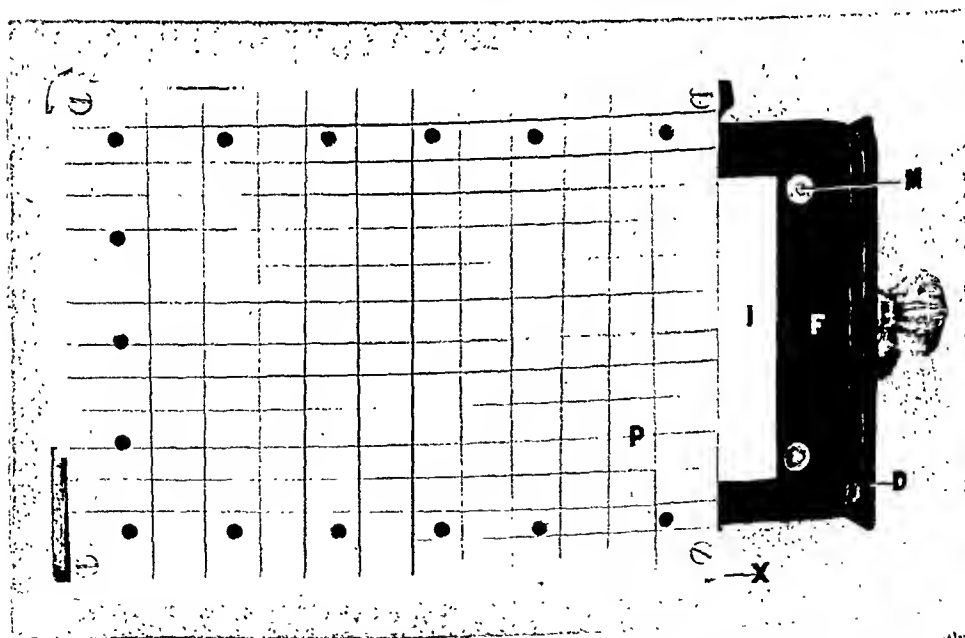


Fig. 2.—Top view of larger freezing attachment, showing aluminum freezing plate and partly open drawer containing a slab of "dry ice."

larger apparatus. The cover of the box is used as the freezing plate. No drawer is used in this case. When the tissue is frozen, the freezing plate is slipped off and the "ice" is removed. The plate is then screwed in place, the box is placed in the object clamp of the microtome, and cutting can begin.

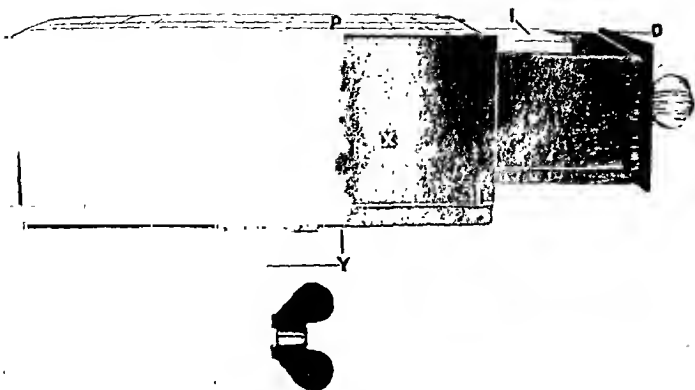


Fig. 3.—Side view of larger freezing attachment, showing bakelite box attached to object clamp supporting base of microtome.

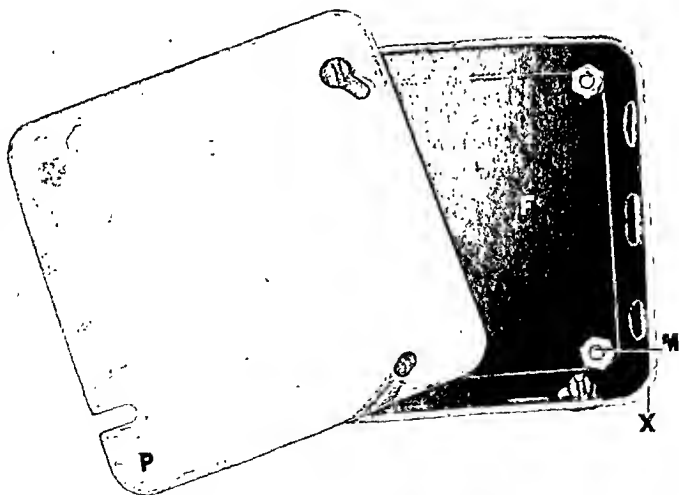


Fig. 4.—Top view of smaller freezing attachment, showing partly removed freezing plate and false bottom within conduit box.

TECHNIQUE

The brain is fixed for ten days in 10 per cent formalin and then cut into slices 1 cm. thick. A slab of brain is placed on the freezing plate and "dry ice" is placed in the freezing chamber. A glass dish is inverted over the tissue. The freezing process varies according to room temperature. Ordinarily the "ice" is removed in about five minutes. The hardening process continues for a few minutes after the "ice" is removed. The surface of the section should be blotted free of water to prevent the formation of ice. Sections 20 to 30 microns thick may be obtained. The sections are brushed into a pan of water where they are straightened out without difficulty. They are easily transferred from dish to dish during the staining process by means of a large perforated flat spoon previously described by Lubinsky.⁴ Further technical details are well outlined in Christeller's *Atlas*⁵ and may be found in Abbott's translation.⁶

ADVANTAGES OF "DRY ICE" METHOD

1. The attachment for freezing coronal sections of the entire cerebrum can be made with materials costing less than \$2.00. The smaller attachment costs about 30 cents. This is a small fraction of the price for a large carbon dioxide gas attachment. No special microtome is required. Using the principles described, one may devise an attachment to suit any microtome.

2. When carbon dioxide gas is used for freezing coronal sections of the cerebrum, a large tank is soon exhausted. "Dry ice" costs only a few cents a pound. A pound of "ice" can be used for freezing two or more coronal sections.

3. The freezing process with "dry ice" requires about five minutes and sectioning may proceed for ten to fifteen minutes. Because of this long interval, some serial sections may be obtained and little tissue is lost in preparatory cutting. According to Christeller, the freezing process with carbon dioxide gas requires twenty to thirty minutes for large sections, and with this method only a few minutes are available for sectioning before refreezing is necessary.

4. The attachment requires little space and is easily transported.

We wish to acknowledge our indebtedness to Dr. Tracy Putnam at whose suggestion this work was undertaken.

REFERENCES

1. Lindsay, W. W., Rice, E. C., and Selinger, M. A.: Frozen Sections Prepared by Use of "Dry Ice," *J. A. M. A.* 96: 773, 1931.
2. Dunn, F. L.: The Use of Solid Carbon Dioxide ("Dry Ice") in the Freezing of Tissues for Microtome Sectioning, *J. LAB. & CLIN. MED.* 16: 627, 1931.
3. Mason, W.: Frozen Sections on Rocking Microtome, *J. Tech. Methods* 19: 86, 1939.
4. Lubinsky, G. W.: Perforated Spoons for Handling Tissue Sections (Frozen, Paraffin, and Pyroxylin), *Arch. Path.* 26: 882, 1938.
5. Christeller, E.: *Atlas der Histotopographie gesunder und erkrankter Organe*, Leipzig, 1927, Georg Thieme.
6. Abbott, M. E.: An Appreciation of Erwin Christeller's *Atlas of the Histotopography of Normal and Diseased Organs*, With an Exposition of His Technique, *J. Tech. Methods* 12: 105, 1929.

AN EFFICIENT OXYGENATOR FOR BLOOD*

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IN AN EARLIER publication¹ a method of introducing oxygen into blood was described. It consisted of producing a thin film of blood on the inner surface of a vertical revolving cylinder. The apparatus was not capable of introducing oxygen rapidly enough for the experimental purposes for which it was designed. In the present report a larger cylinder of improved design, capable of introducing 60 per cent more oxygen into blood, is described.

The principal feature of the apparatus is a vertical steel cylinder, 24 inches in length and $8\frac{1}{2}$ inches in inside diameter (Fig. 1). The cylinder is supported by two sets of roller bearings with circular racers. These allow the cylinder to revolve around a vertical axis. The racers are in turn supported by a vertical steel pipe 4 inches in diameter, with a wall $\frac{5}{8}$ inch thick. The pipe is 40 inches high, mounted on a steel base, 12 by 20 inches in area. Revolution of the cylinder is accomplished by a one-quarter horse power A.C. motor, attached by a pulley and V-belt to a vertical segment of a steel sphere covered with hard rubber (Fig. 1, side view). The convex surface of the segment of a sphere is pressed by a strong steel spring against the beveled edge of a steel collar that encircles the revolving cylinder. Revolution of the spherical segment produces revolution of the cylinder by this sliding friction drive that has a contact of about one-half inch. The spherical steel segment can be moved about a horizontal axis in such a way as to shift the frictional driving surface from the center to the periphery of the spherical segment. This shift is capable of varying the speed from 60 to 360 r.p.m. This is an improvement upon the previous method¹ of changing the rate of revolution of the cylinder by varying the armature resistance of a D.C. motor.

In the old apparatus great difficulty was experienced in producing a rust-proof inner surface for the cylinder. In the present apparatus a "Micarta"† cylinder $\frac{1}{8}$ inch thick fits snugly within the steel cylinder and provides a smooth, rust-proof surface for the film of blood. The lower end of the cylinder is tapered from within outward to a knife edge. A chrome-plated copper cup slides up over the lower end of the cylinder, overlapping the cylinder by $1\frac{1}{2}$ inches. The space between the lower, sharp end of the cylinder and the inner wall of the stationary cup is less than 1 mm. The lower part of the cup is funnel-shaped and terminates in a central aperture $\frac{3}{4}$ inch in diameter, from which a tapered

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†A plastic made by Westinghouse Electric Company.

collar extends downward. Over this tapered collar is slipped the neck of a glass cup with a similar taper. The junction between the ground-glass neck and the metal collar is sealed with stopcock grease, and the glass cup is held in position by two small springs extending from the glass cup to the bottom of the metal cup. Both the metal and the glass cups are jacketed separately to permit the circulation of water between the walls in order to maintain the blood at the desired temperature.

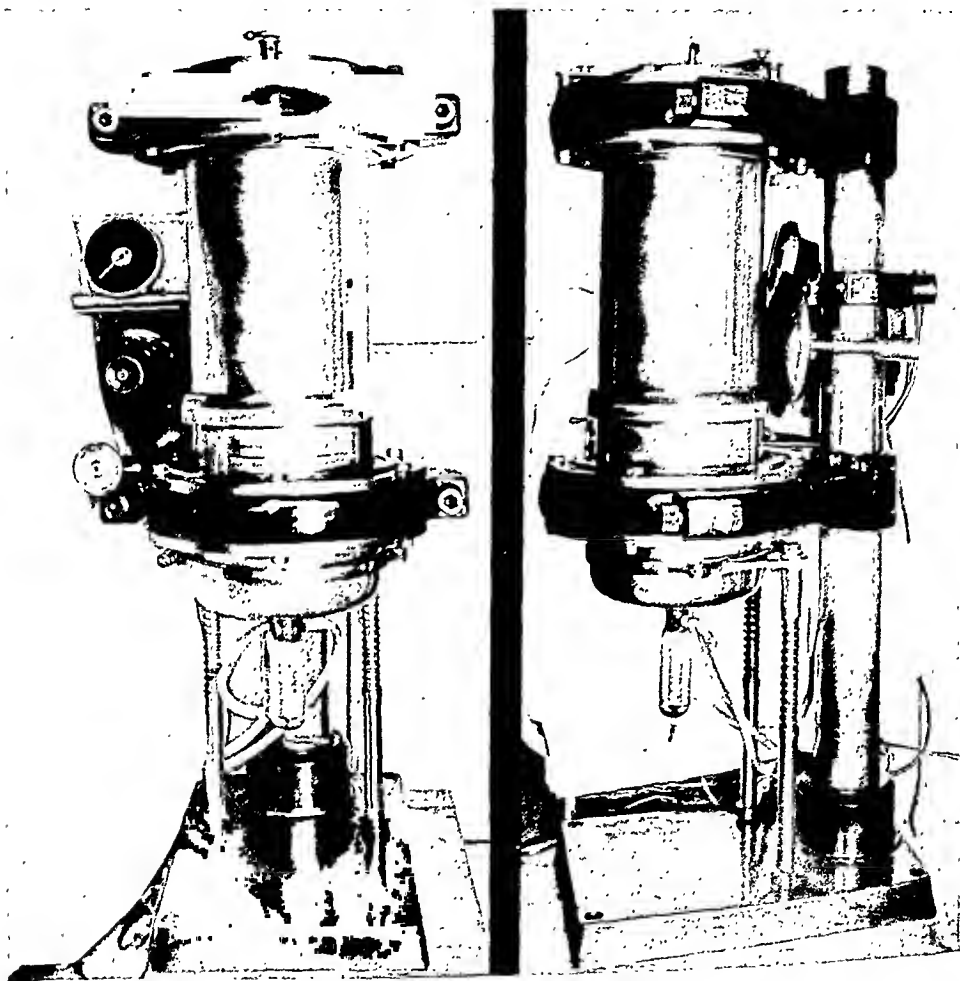


Fig. 1.—Photographs of oxygenator. Front and side views.

The greater portion of the space within the revolving cylinder is occupied by a closed, stationary cylinder, as in the previous model. Through the center of this closed cylinder passes a tube which is used to convey a mixture of oxygen and carbon dioxide at a rate of 5 liters per minute to the lower portion of the inside of the apparatus. The oxygen mixture then diffuses upward in close contact with the inner wall of the revolving cylinder. When using the apparatus for perfusion, a mixture of 95 per cent oxygen and 5 per cent carbon dioxide is used so as to maintain an approximately normal carbon dioxide content in the blood.

Blood is introduced at the top of the cylinder through a stainless steel tube having a 2.5 mm. bore. The tube is curved and its end is flattened so that it delivers a fanlike stream of blood against the inner wall of the revolving cylinder in the direction of revolution and tangential to the curved surface of the cylinder. The tube is locked in position on the inner stationary cylinder, but is detachable for cleaning. When blood is pumped through this inlet tube onto the upper part of the inner surface of the cylinder while it is revolving, a film of blood is established in a horizontal band about 4 inches high around the upper part of the cylinder. At the lower margin of this band the film of blood breaks up into vertical rivulets of blood approximately 0.5 cm. in width, spaced at intervals of 2 cm. Under these circumstances relatively small amounts of oxygen are introduced into the blood. Consequently, it was found necessary to prime the cylinder with blood in order to establish a film. This priming can probably be accomplished in a variety of ways. It was found simplest to wipe the vertical length of the cylinder with a gauze sponge while the cylinder is revolving and the blood is flowing in at the top. Once the film is established, it will remain as long as the cylinder is revolving and blood is flowing in at the top; but both the revolution of the cylinder and the flow of blood may be stopped simultaneously for brief intervals without the loss of the film.

The optimum rate of revolution of the cylinder is in the neighborhood of 250 r.p.m. Faster rates have not facilitated the introduction of oxygen into blood. With slower rates the film of blood on the inner surface tends to split up into rivulets when the flow of blood through the oxygenator is small. Foaming occurs only if air enters the tubing through which blood is being pumped into the oxygenator.

INTRODUCTION OF OXYGEN

The amount of oxygen that can be added to blood by this apparatus was determined in the following manner: Venous blood was continuously withdrawn from the superior vena cava of an animal and pumped into the top of the oxygenator. The oxygenated blood was pumped back from the cup at the bottom of the cylinder into the femoral artery in a central direction. The pumps used were of the constant injection roller type as modified by DeBakey² and driven by an electric motor.³ The rate of the pumps was varied by an armature resistance, and the speed of the motor was read directly on a speedometer. The output of the pumps was calibrated by measuring the delivery of blood at different rates of revolution after the experiment. Cats were used in five instances, Nos. 1 to 5, inclusive, in Table I; and dogs in six instances, Nos. 6 to 11. Several weeks prior to the experiments on the cats, the ribs overlying the pericardium were excised and the pericardium was sutured to the chest wall just beneath the skin. The pulmonary artery could thus be exposed and clamped without producing an open pneumothorax. In all the cats the pulmonary artery was completely occluded. During the period of occlusion the circulation of blood through the animal was maintained by the mechanical pumps, and the respiratory function of the lungs was performed by the oxygenator.³ To obtain a larger flow of blood dogs were used in the last six observations in Table I. In these animals

the pulmonary artery was not occluded. In observations Nos. 8, 9, 10, and 11, the dogs were allowed to inhale from a spirometer filled with 85 per cent nitrogen and 15 per cent oxygen in order to decrease the saturation of the venous blood with oxygen. In all the experiments sufficient heparin was given intravenously to prevent coagulation of the blood. Simultaneous samples of blood were withdrawn under oil from the tubes leading to and from the oxygenator. The samples were immediately analyzed for oxygen and carbon dioxide content and oxygen capacity. The determinations were made in duplicate. The greatest difference between duplicate samples in oxygen content was 0.15 volumes per cent, and in oxygen capacity it was 0.20 volumes per cent.

TABLE I

OBSERVATION NO.	OXYGEN CAPACITY (VOL. %)	SATURATION		OXYGEN ADDED TO BLOOD (C.C./100 C.C.)	BLOOD FLOW (C.C./MIN.)	OXYGEN ADDED TO BLOOD (C.C./MIN.)	CARBON DIOXIDE IN OXYGEN (%)
		% BEFORE	% AFTER				
1	16.75	57	99	6.88	130	8.94	5
2	17.01	36	99	10.57	240	25.37	0
3	14.51	58	93	5.19	255	13.23	6
4	14.76	72	95	3.52	270	9.50	5
5	14.23	41	99	8.35	285	23.80	5
6	17.32	72	96	4.11	370	15.21	5
7	21.90	73	92	4.24	375	15.90	5
8	21.19	48	82	7.39	380	28.08	5
9	22.03	40	72	7.04	395	27.81	5
10	17.13	32	64	5.41	420	22.72	7
11	21.87	64	95	6.71	455	30.53	5

The results are arranged in Table I in order of increasing blood flow. It can be seen that up to rates of 375 c.c. per minute the blood leaving the oxygenator is adequately saturated with oxygen at varying degrees of venous saturation. In three instances (Nos. 8, 9, 10) the saturation of the blood leaving the oxygenator was below 92 per cent. In one of these, No. 10, the blood was only 64 per cent saturated, but there was marked venous unsaturation; a gas mixture of 93 per cent oxygen and 7 per cent carbon dioxide was passed through the oxygenator instead of the usual mixture of 95 per cent oxygen and 5 per cent carbon dioxide. In this experiment the carbon dioxide content of the blood leaving the oxygenator was 51.23 volumes per cent, whereas in the other ten instances the carbon dioxide content varied between 42.83 volumes per cent and 30.59 volumes per cent. In Nos. 8 and 9 there was also a marked unsaturation of venous blood, and the flow was relatively large.

HEMOLYSIS

To maintain normal function when perfusing an isolated kidney it is probably important to avoid hemolysis in the blood used for perfusion.⁴ Similarly, in the perfusion of the entire body of an animal it must be equally important to avoid hemolysis. To determine the degree of hemolysis produced by the oxygenator, blood was allowed to flow into the apparatus by gravity from a 250 c.c. burette at a rate between 250 and 350 c.c. per minute (the range of blood flow

used to perfuse cats of average size). Blood was drained from the glass cup at the bottom of the oxygenator into a flask which was intermittently emptied into the burette which supplied blood to the oxygenator. The total volume of blood used in these observations was approximately 600 c.c. The temperature of the blood was maintained between 37.5° and 38.5° C. by the circulation of warm water through the double walls of the stationary metal and glass cups.

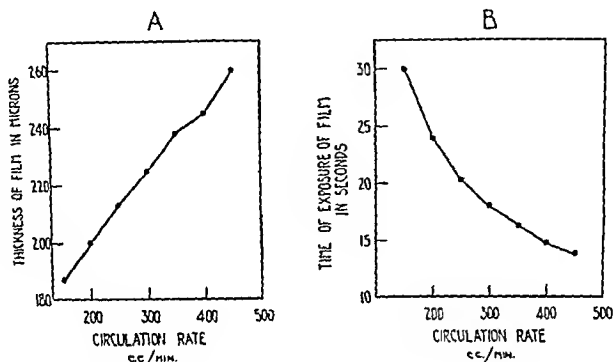


Fig. 2.—A, Relation of flow of blood through oxygenator to thickness of blood film. B, Relation of blood flow to time of exposure of film.

The blood used was obtained from a dog the day before the observations were made, and was kept in an icebox overnight. Coagulation was prevented by the addition of heparin. At the start of the observations, a sample of blood was placed in a Wintrobe hematocrit tube.² The blood was then passed continuously through the oxygenator with the cylinder revolving at 250 r.p.m. Samples of blood were removed and placed in hematocrit tubes every fifteen minutes, until the blood had circulated through the oxygenator for an hour and a quarter. Cells were separated from the plasma in the hematocrit tubes by centrifuging. The colors of the samples of plasma were then compared with the colors of a set of standard tubes of similar diameter containing known dilutions of completely hemolyzed red blood cells. It was found that up to thirty or forty-five minutes the plasma remained practically clear (i.e., faint straw color). From then on there was a steadily increasing rate of hemolysis. At the end of an hour and fifteen minutes of passage through the oxygenator, the plasma had a pronounced reddish tinge.

THE BLOOD FILM

The area available for the filming of blood on the inner surface of the vertical revolving cylinder is 4,000 sq. cm. This represents a 58 per cent increase in area over the oxygenator previously described.¹ The glass cup at the bottom of the oxygenator holds 12 c.c. of blood at an appropriate operating level. The volume of blood on the surface of the revolving cylinder was determined at different rates of blood flow as follows: A known quantity of blood

was placed in a graduated glass cylinder. The blood was then pumped from this cylinder into the copper cup at the bottom of the oxygenator from which it drained down into the glass cup. From the glass cup it was pumped back into the graduated glass cylinder, maintaining at all times a constant level of blood in the glass cup. The level of the blood in the graduated glass cylinder was then read at different rates of blood flow from 150 to 450 c.c. per minute. The volume of blood contained in the circuit at different rates of blood flow was thus determined. The revolving cylinder was then added to the circuit, and the determinations were repeated. In this manner the volume of blood on the sides of the revolving cylinder was determined at varying rates of blood flow. From these figures the thickness of the film and the time of exposure of the blood to oxygen were calculated. For example, 85 c.c. of blood are distributed over the surface of the oxygenator when the cylinder is revolving at 250 r.p.m. and blood is flowing through the apparatus at the rate of 250 c.c. per minute. Under these conditions the film of blood on the inner surface of the revolving cylinder is 213 microns thick, and the average time of exposure of the blood film to oxygen is 20.4 seconds.

The graphs of Fig. 2 summarize the observations made. Each point on the graphs represents the mean of six determinations. It was found that with rates of flow from 150 to 450 c.c. per minute the depth of the blood film was directly proportional to rate of blood flow (Fig. 2A). The time the film of blood was exposed to oxygen on the inner surface of the revolving cylinder decreased, but at a progressively diminishing rate, as the flow of blood through the apparatus increased from 150 to 450 c.c. per minute (Fig. 2B). Both the greater depth of the film and the decreased time of exposure will tend to interfere with the absorption of oxygen by the blood. Therefore, at any given unsaturation of the blood entering the oxygenator, it is to be expected that, as the rate of blood flow increases, a point will be reached at which the blood leaving the oxygenator will no longer be adequately saturated with oxygen. That such a point has not been reached with degrees of venous oxygen unsaturation within the normal range at rest and a blood flow as great as 450 c.c. per minute is indicated by observation No. 11, Table I.

SUMMARY

An oxygenator capable of introducing as much as 30 c.c. of oxygen per minute into blood has been described. The apparatus is designed to accommodate rates of blood flow from 100 to 500 c.c. per minute. Up to 300 c.c. per minute, the blood leaving the oxygenator will be adequately saturated with oxygen even with marked degrees of venous unsaturation; and at rates of flow up to 450 c.c. per minute if the venous unsaturation is within the normal range. The apparatus is simple in construction and easy to operate. Foaming of the blood does not occur, and hemolysis is slight in periods up to forty-five minutes of operation.

REFERENCES

1. Gibbon, J. H., Jr.: An Oxygenator With a Large Surface-Volume Ratio, *J. LAB. & CLIN. MED.* 24: 1192, 1939.

2. DeBakey, M. E.: Simple Continuous-Flow Blood Transfusion Instrument, New Orleans M. & S. J. 87: 386, 1934.
3. Gibbon, J. H., Jr.: The Maintenance of Life During Experimental Occlusion of the Pulmonary Artery Followed by Survival, Surg., Gynec. & Obst. 69: 602, 1939.
4. DeGowin, E. L., Warner, E. D., and Randall, W. L.: Renal Insufficiency From Blood Transfusion. II. Anatomic Changes in Man Compared With Those in Dogs With Experimental Hemoglobinuria, Arch. Int. Med. 61: 609, 1938.
5. Wintrobe, M. J.: A Simple and Accurate Hematocrit, J. Lab. & Clin. Med. 15: 287, 1929.

A DEVICE FOR FIXING THE BRAIN AND SPINAL CORD IN ONE PIECE*

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AFTER removing the brain and spinal cord as a single specimen, it is often found difficult to restore them in their proper relationships. In an ordinary brain jar, or even in a larger receptacle, the spinal cord frequently becomes twisted and its anatomic features are distorted. The following simple apparatus was devised to obviate this difficulty:

A glass tank (the type used for household aquariums are easily obtained and relatively inexpensive), 25 by 10.5 by 10 inches in size, is used as a receptacle for primary fixation. A glass cover is fitted over the top of the tank. A metal framework, consisting of two separate parts, as pictured in Fig. 1, is placed within this container which has been partially filled with 10 per cent formalin. The purpose of the framework is to provide a suitable crossbar at either end of the tank from which a brain may be suspended and hang freely, and two properly placed grooves along which the respective spinal cords may be extended in proper alignment with the given brain.

The framework is constructed from two pieces of galvanized wire, $\frac{1}{8}$ inch in diameter and 130 and 95 inches in length, respectively. These are bent in a continuous pattern, as seen in Fig. 2, with the aid of vise, pliers, and hammer. Each division of the framework is 7 inches high and 9 inches wide, and provides a series of crossbars resting on uprights, approximately $3\frac{1}{2}$ inches apart. Each crossbar except the first has two grooves an inch wide and an inch deep which lie approximately $1\frac{1}{4}$ inches apart. These are the grooves in which the spinal cords are supported. The two divisions of the framework are so devised as to form one continuous pattern when placed end to end in the tank. In this fashion, the complete framework has one ungrooved crossbar available at each end of the tank, from which a brain may be suspended and hang freely by means of a loop of thread or metal hook. The respective cord of each of the brains may then be placed in the two grooves of the framework and thereby kept in good alignment with the brain.

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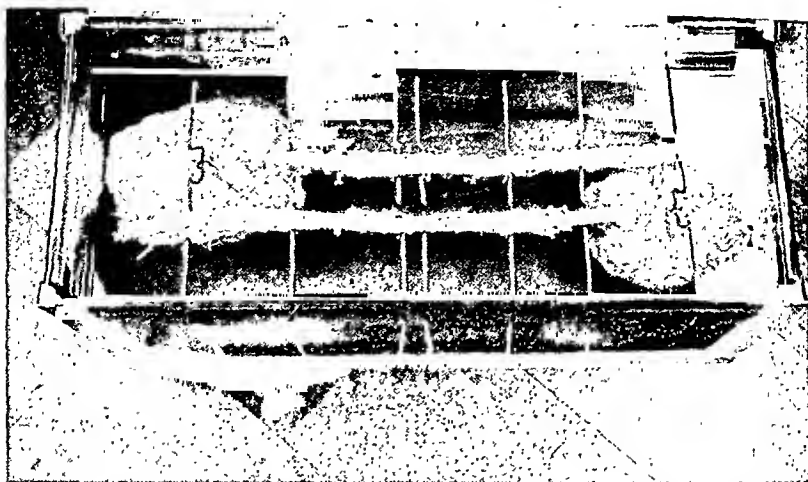


Fig. 1.—Glass household aquarium with brains and their attached spinal cords supported in grooves of wire framework.



Fig. 2.—Framework consisting of two lengths of wire bent into continuous pattern.

The brain is shifted on the crossbar, so that the spinal cord lying in the groove forms a continuous column with the brain stem. Having the framework in two portions permits the removal of one of the two brains without disturbing the other, or the addition of a fresh specimen to the tank when one is already in place.

A RAPID METHOD FOR STAINING BLOOD SMEARS IN DETERMINING OPSONOCYTOPHAGIC INDICES*

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BECAUSE of the unsatisfactory results obtained from staining blood smears for opsonocytophagic indices with Hasting's stain as recommended by Huddleson and co-workers,⁵ many new methods have recently been reported (Cape and Jaffe²; Flinn⁴; Bruner and Edwards¹; Calder³). Satisfactory staining for this work, in addition to being simple, should permit accurate enumeration of the organisms ingested by phagocytes. Polymorphonuclear neutrophils must be readily recognized, and phagocytized bacteria must not be mistaken for cytoplasmic granules. Some of the newer methods (Flinn⁴), while satisfactory, require stains or reagents which are not usually available to most routine laboratories.

The method reported here has been used in this laboratory with considerable success. Citrated blood is mixed with a saline suspension of *Brucella* cells in the usual manner and incubated at 37° C. for thirty minutes. Blood smears are made and dried quickly to prevent shrinkage of white blood cells. These are then put through the flame of a Bunsen burner four times and allowed to cool. The fixing of smears by flaming is very important and may require practice before the right amount of flaming is obtained. If not flamed sufficiently, smears will tend to wash off the slide, whereas overflaming will result in smears too deeply stained.

The fixed smears are covered with 10 per cent Ziehl-Neelsen carbolfuchsin. This stain is prepared by diluting $\frac{1}{10}$ the Ziehl-Neelsen carbolfuchsin used for tubercle bacillus staining with distilled water and filtering. At the end of two minutes the slides are washed gently with tap water to prevent the dye from precipitating on the slide, and then allowed to dry.

By this method *Brucella* and the nuclei of the phagocytes stain deep red. The cytoplasm of the latter is a light pink; granules are not stained. Red blood cells stain weakly. Phagocytized bacteria appearing against the lightly stained cytoplasm are easily counted.

This method has also proved satisfactory for phagocytosis studies with streptococci, staphylococci, pneumococci, *H. influenzae*, and *H. pertussis*.

I wish to acknowledge with gratitude the advice of Dr. John A. Kolmer who suggested the use of carbolfuchsin.

REFERENCES

1. Bruner, D. W., and Edwards, P. R.: The Application of an Endospore Stain to Blood Smears From Opsonocytophagic Tests, *J. LAB. & CLIN. MED.* 25: 543, 1940.
2. Cape, H. E., and Jaffe, H.: A New Stain for Opsonocytophagic Tests, *Am. J. Clin. Path.* 9: 41, 1939.
3. Calder, R. M.: Modification of Huddleson's Opsonocytophagic Reaction, *J. LAB. & CLIN. MED.* 25: 769, 1940.
4. Flinn, M.: A Rapid Staining Method for Opsonocytophagic Indices, *J. LAB. & CLIN. MED.* 25: 316, 1939.
5. Huddleson, I. F., Johnson, H. W., and Hamann, E. E.: Study of Opsonocytophagic Power of Blood and Allergic Skin Reactions in *Brucella* Infection and Immunity in Man, *Am. J. Pub. Health* 23: 917, 1933.

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THE STANDARDIZATION OF CERTAIN FACTORS IN THE CUTANEOUS "VENOSTASIS" BLEEDING TIME TECHNIQUE*

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UNTIL all the factors concerned in hemostasis are known and can be tested individually by adequately quantitative methods, the determination of bleeding time will remain a necessary and valuable test of the "bleeding tendency." A disturbance of hemostasis obviously involves defects in either the process of coagulation, or the capillaries, or a combination of both. The presence or absence of a "bleeding tendency" would not necessarily be revealed even if one were to test for a coagulation defect by adequate methods and for capillary fragility by the suction or tourniquet technique. It is possible that a defect in coagulation might be neutralized by a compensatory response in the capillaries or vice versa. In other situations, however, the imperfections of one might augment those of the other. It is desirable, therefore, both for physiologic experimentation and for clinical purposes, to have a reliable method for the determination of bleeding time in order to study the end result of the relationships between the defects in coagulation and in the capillaries or peripheral tissues.

For this reason, we have undertaken a study of the reliability and limitations of the venostasis bleeding time test.^{1, 2} This test was selected because venostasis usually prolongs the bleeding time of normal subjects¹ and hence tends to accentuate differences and defects that otherwise might be missed.

The tests reported in this study were performed exclusively on the bleeding tendency of the skin. It should be remembered that the results are not necessarily applicable to the mucous membranes and other tissues.³

EXPERIMENTAL

The Lancet.—The Sharpe and Smith lancet was used. It was selected because we believed the wide base might serve to distribute the pressure more equally. In a preliminary study it was found that setting the blade length at 2.5 mm. below the level of the rim of the base resulted in too great a percentage incidence of "no bleeding." The use of a 3.0 mm. blade length decreased but did not abolish the number of "no or zero bleeding" cuts.

Another difficulty was the sharpness of the blade. Cuts made with a sharp blade bleed more freely than those made with a dull blade. The blades were kept sharp with very fine pumice and a leather strap. If sharpened too frequently, the metal is worn away so that the thicker portion of the blade remains.

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New lancets in a single shipment were found to be well standardized as to width, but not as to thickness. Ideally, thin and replaceable blades, as in the Tocantins' instrument,³ should be employed.

Control of Pressure.—Standardization of the depth of the puncture is one of the most essential features of a good bleeding time test. It seemed probable that control of the pressure placed upon the lancet before the blade is released might contribute to the uniformity of the punctures.

In order to control pressure, the lancet was mounted on a framework to which an arm rest was attached. The sliding bar which held the lancet was so weighted that when the lancet was placed on the average arm, its rim depressed the skin approximately 1 cm. This amount of pressure gave exceptionally short bleeding times in subjects with a well-developed pronator muscle and little subcutaneous fat. In these cases the skin was not depressed 1 cm. by the weighted lancet, and it became evident that a constant pressure did not give the desired uniformity of the punctures. We, therefore, devised a gauge for the lancet that would indicate when the skin was depressed exactly 1 cm., and when necessary manual pressure was added till that depth was reached. This procedure resulted in unduly long bleeding times in the very muscular subjects. It is obvious, then, that neither the application of the same amount of pressure in all cases nor the identical depression of the peripheral tissues was able to insure punctures of equal depth.

With the mechanical refinements used, the results showed a slight decrease in variability as compared with those obtained using the ordinary clinical procedure. However, the improvement was within the experimental error and hence was not significant. From this we conclude that with experience one can acquire a judgment in regard to pressure that is about as accurate as these mechanical devices for the production of uniform punctures.

The area of skin in which the punctures were made. With the subject seated and his forearm (left) in the supine position, the area over the pronator (lateral aspect of forearm below the cubital fossa) was washed gently with 95 per cent alcohol. (We have found that either forearm may be used.) The lancet was sterilized with alcohol and dried. After any hyperemia that may have occurred from the alcohol wash had disappeared, the pressure in a sphygmomanometer cuff about the arm was raised to 40 mm. Hg. A hemostat was used to prevent leakage of air from the cuff through the inflation bulb. The puncture or punctures were then made.

Since we were interested in comparing bleeding times in the same individual, it was necessary to make a number of punctures. Five series of two punctures were made on our 88 subjects as follows: with the pressure in the cuff at 40 mm. Hg, a puncture was made, and then five seconds later a second puncture was made, not less than one inch from the first puncture. The blood collected at each puncture was blotted with filter paper at ten-second intervals, timed with a stop watch. The filter paper was not pressed so firmly as to open the wound. The bleeding time is easily determined by multiplying the number of drops of blood on the filter by ten seconds.

After bleeding from the two punctures had ceased, the cuff was deflated and the arm was very mildly exercised. Then two more punctures were made an inch apart. This procedure was repeated until the bleeding time of five sets of two punctures each had been determined. This provided the data for analysis.

Subjects.—Eighty-eight subjects, 58 men and 30 women, of medical school age were used. Retests were made on 31 subjects, some of them being tested on several occasions.

Three additional subjects were excluded from this study because their mean bleeding times were definitely abnormal (*vide infra*). For some unknown reason an occasional subject continued to ooze blood-tinged serum for a relatively prolonged period. Data from such subjects were not used.

RESULTS AND DISCUSSION

The "*clinically normal*" maximum venostasis bleeding time from a single puncture. In Fig. 1 the 1,280 readings on the 88 subjects are distributed according to frequency. The bleeding times ranged from 0 to 310 seconds. The upper limit for "*the clinically normal*" found by Ivy, Shapiro, and Melnick¹ was 240 seconds. According to our results, 240 seconds includes 99.54 per cent of the readings. It is our belief that the few bleeding times above 240 seconds were due to striking a hidden vein because they were not typical of the other bleeding time measures obtained from the same individuals. Thus, 240 seconds as a "*clinically normal*" maximum venostasis bleeding time for a "*one puncture test*" is confirmed for the age group studied, unless a vein is accidentally punctured.

In setting a "*clinically normal*" maximal "*venostasis*" bleeding time at 240 seconds on the basis of a "*one puncture test*," patients with a mild to moderate "*bleeding tendency*" may be diagnosed as normal. This is likely because of the errors inherent in any relatively crude method. However, the errors usually tend to cancel each other when a number of tests are made. Hence the mean of several bleeding times more closely represents the true status.

The "*clinically normal*" mean or average venostasis bleeding time. In Fig. 1 the mean bleeding times of the 88 subjects are distributed according to frequency. The mean bleeding time of the subject who had the best cutaneous hemostatic mechanism by this test was twenty seconds, and that with the poorest was 125 seconds. Thus, a patient would be "*clinically abnormal*" if the mean bleeding time as determined by the test outlined above was significantly greater than 125 seconds.

COMMENT

From the preceding discussion of the results the advantage of making more than one test of bleeding time is obvious. However, the patient and the time factor have to be considered. The question then arises: What is the least number of punctures required to yield a reasonably reliable mean cutaneous venostasis bleeding time? A subsidiary question is: When two punctures are made, one within five seconds of the other, does the making of the first puncture modify significantly the bleeding time of the second puncture?

What is the least number of determinations required to yield a reasonably reliable cutaneous "*venostasis*" bleeding time? In Table I are listed the various

means and medians that may be used as a bleeding score. Medians were proposed to eliminate extreme readings which weight the means.

As can be seen from Fig. 1 the frequency of zero bleeding times is greater than that of any other bleeding time. Table II gives the distribution of the zero readings over the ten successive punctures. Due to the influence the zeros would have on the variability of the means or medians, these were recalculated using the first 3, 4, or 6 individual punctures that bled instead of the first 3, 4, or 6 punctures made (Table I).

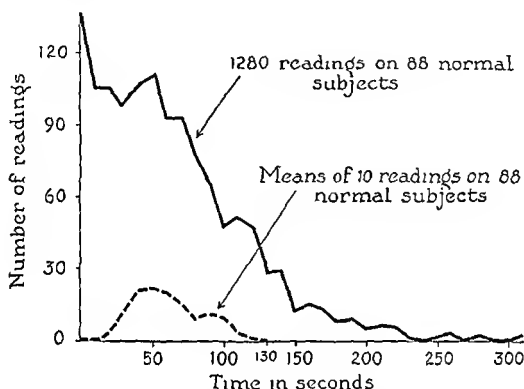


Fig. 1.—Distributions of bleeding times
A = 1,280 readings on 88 normal subjects
B = Means of 10 readings on 88 normal subjects.
Abscissae = Time in seconds
Ordinates = Number of readings

Regarding each of the listed means and medians in Table I as the result of a proposed bleeding time test, four criteria will be used to examine their relative value: (1) their similarity to the mean of 10; (2) their reliability as judged by the test-retest method; (3) their reliability as judged by the deviations from their means; and (4) the practical consideration which calls for the shortest test possible.

The deviations of each measure from the mean of 10 are shown in Table I, and the significance of the differences is indicated by the critical ratios. The mean of the first three odd punctures that bled is almost exactly the same as the mean of 10, and the median of the first four that bled shows only a slight difference. Since a critical ratio must be 2.60 or more to indicate a statistically significant difference with this number of cases,⁴ it may be seen that the mean of the first 6 that bled, and the mean of the middle 6 are significantly larger than the mean of 10. The mean and the median of the first 4 made, and also the median of the first 3 made are significantly smaller than the mean of 10. The directions of these deviations suggest that the exclusion or preponderance of zero scores is responsible for the extent of variation.

The correlation of each of these measures with the mean of 10 is another demonstration of similarity. All the correlations are spuriously high because the individual readings from which each of the smaller means or medians is derived are also included in calculating the mean of 10. However, the correlations will not misrepresent the facts if the relationships are interpreted as showing the similarity of 128 bleeding time scores computed by each of two methods, and not as indicating the accuracy with which, knowing the mean of 10, one could predict the compared mean or median that would be obtained if the necessary six, four, or three additional punctures were made.

TABLE I

MEASURES OF BLEEDING TIME DERIVED FROM VARIOUS NUMBERS OF PUNCTURES. THE COMPARISON OF EACH WITH THE MEAN OF 10 PUNCTURES, AND THE SELF-CORRELATION OF EACH AFTER A RETEST

MEASURE OF BLEEDING TIME	TIME IN SECONDS	CORRELATION WITH MEAN OF 10	DIFFERENCE FROM MEAN OF 10	C.R.* OF DIFF. FROM MEAN OF 10	CORRELATION TEST-RETEST
Mean of 10 punctures	61.56 ± 2.1				+0.30
Mean of first 6 made	59.69 ± 2.3	+0.90	1.87	1.85	+0.34
Mean of first 6 that bled	66.95 ± 2.3	+0.87	5.39	4.73	+0.13
Mean of first 4 made	56.64 ± 2.7	+0.74	4.92	2.68	+0.30
Mean of first 4 that bled	65.23 ± 2.5	+0.77	3.67	2.25	+0.17
Median of first 4 made	53.28 ± 2.8	+0.74	8.28	4.40	+0.36
Median of first 4 that bled	62.19 ± 2.6	+0.73	0.63	0.35	+0.36
Median of first 3 made	48.28 ± 2.6	+0.69	13.28	6.95	+0.55
Median of first 3 that bled	58.13 ± 2.5	+0.65	3.43	1.72	+0.29
Mean of middle 6 made	64.06 ± 2.6	+0.94	2.50	2.61	+0.29
Mean of first 3 odd that bled	61.41 ± 2.4	+0.91	0.15	0.15	+0.33

$$*C.R. = \text{Critical ratio} = \frac{\text{Obtained difference}}{\text{Sigma of difference}}$$

$$\text{Sigma difference} = \sqrt{(\sigma m_1)^2 + (\sigma m_2)^2 - 2r\sigma m_1\sigma m_2}$$

r = Coefficient of correlation

Obviously, each addition of identical data raises a correlation. Therefore, the means of 6 would be expected to show the highest correlation in this particular situation. *It is thus of great interest to note that the mean of the first 3 odd punctures that bled has a correlation as high as the means whose correlations involved twice as many identical readings.*

The reliability of a test shows the degree of consistency with which it measures. One indication of reliability is a high correlation between two sets of results when, under controlled conditions, the test is repeated on the same individuals. Unfortunately, this "test-retest" information is available for only 31 cases. The correlations are given in Table I; they are all unduly low because the narrow limits of the data included in this study do not permit the range of variability between one individual and another which is necessary for any test to demonstrate its maximum value. Therefore, the significance of the "test-retest" correlations is for comparative purposes only. It will be noted that the zero scores are again a determining factor, since the highest correlation is the median of the first 3 punctures made, where the incidence of zero readings predominates, and the correlations of the means of data from which the zeros are omitted, are conspicuously inferior.

There was no consistent trend toward a longer or shorter bleeding time when the test was repeated on 31 subjects. The mean bleeding times of the 31 first tests were about the same as the means of the 31 repeat tests. The means or medians of 10, 6, 4, or 3 punctures for the 31 cases varied from 50 to 65 seconds in both the first and repeat tests. It was apparently mere chance as to which was the higher, each one being slightly superior as gauged by about half of the measures. As indicated by the low correlations (Table I) there was little consistency between the first and repeat tests of each individual. This is to be expected with such a homogeneous group.

TABLE II
INCIDENCE OF ZERO READINGS

CONSECUTIVE PUNCTURES	1	2	3	4	5	6	7	8	9	10
% of total (128 cases)	20	19	9	9	11	10	8	8	6	5
% of men (53 cases)	21	16	10	8	7	16	12	8	5	7
% of women (30 cases)	19	21	9	10	15	5	5	7	8	5

Another criterion of reliability is a small standard error or deviation of the mean of a series of tests. The standard error, or sigma, of a mean (σ_m) is a measure of variability which indicates the range within which a "true mean," or the mean of an infinite number of cases, would have a 67 per cent chance of falling. It is also the range within which 67 per cent of the means would fall if other sample groups of the same size were tested under conditions identical to those producing the original mean. Therefore, if the range designated by the sigma of a mean is small, the test is consistent.

The standard errors of the means and medians listed in Table I were obtained by assigning to each person a bleeding time based on the mean of 10 punctures or the median of 3, etc., and finding the sigmas of the mean of the 128 bleeding times so defined. They are not strikingly dissimilar. It may, therefore, be concluded that if one wishes to determine the average bleeding time of a group of about a hundred people, any of the proposed tests would offer approximately the same degree of reliability. However, there would be a trend in the direction of greater consistency in the measures whose individual scores were based on a larger number of punctures. An exception to this trend is noted in favor of the mean of the first 3 odd punctures that bled.

This material on standard errors of the means or medians of 128 cases gives no indication of the reliability of the various means or medians for determining the bleeding time of individual persons, which is of much greater clinical importance than the measurement of groups. *The standard error of a mean or median of the punctures made on any subject is the range within which the chances are 2 to 1 that his "true bleeding time" would lie if established by an infinite number of punctures.* Naturally this range differs in individuals. By finding the average standard error of the 128 means and medians, a comparison can be made of their reliability as individual bleeding time tests. The mean standard errors are listed in Table III, together with their correlations and differences from the average sigma of the means of 10.

The formula for deriving a sigma of the mean being

$$\frac{\text{Sigma of distribution}}{\sqrt{\text{Number of cases}-2}}$$

when there are 10 cases, and

$$\frac{\text{Sigma of distribution}}{\sqrt{\text{Number of cases}-3}}$$

when there are less than 10, it can be seen that the measures based on fewer than 10 punctures were heavily penalized.⁵ It may be argued that such an inequitable comparison is of little value, but this is not the case, since the issue will be encountered every time one investigates the significance of a difference between bleeding measures made under varied conditions, as, for example, before and after treatment.

TABLE III

RELIABILITY OF THE MEASURES AS GAUGED BY INTERNAL CONSISTENCY

MEASURE OF BLEEDING TIME	MEAN SIGMA OF MEANS	DIFFERENCE FROM SIGMA MEAN OF 10	C. R.	CORRELATION WITH SIGMA MEAN OF 10
Mean of all 10	14.73 ± 0.51			
Mean of first 6 made	22.51 ± 0.92	7.78	14.88	0.80
Mean of first 6 that bled	17.15 ± 0.86	2.42	5.69	0.96
Mean of first 4 made	31.09 ± 1.54	18.61	12.48	0.57
Mean of first 4 that bled	29.57 ± 1.53	14.84	13.86	0.60
Median of first 4 made	33.40 ± 1.60	18.67	13.24	0.51
Median of first 4 that bled	31.72 ± 1.60	16.99	12.67	0.62
Median of first 3 made	31.33 ± 1.94	16.60	9.70	0.56
Median of first 3 that bled	29.02 ± 1.84	14.29	8.82	0.53
Mean of first 3 odd that bled	25.31 ± 1.71	10.58	6.82	0.45

The differences between the mean sigmas of the means of 10 and the mean sigmas of all the other measures are statistically significant. The large critical ratios are due to the spuriously high correlations (see footnote to Table I). It can be seen by inspection of the table that except for the sigmas of the means of six punctures, the differences between any two of the other measures would not be significant unless equally high correlations exist between them. The sigmas of the means of the 3 odd punctures might also be an exception. However, statistical analyses were not made to confirm this.

The standard errors of the means and medians of individual cases are open to criticism from a statistical standpoint because the formulas from which they are calculated are based on the assumption that the data fall into a "normal distribution curve." The curve obtained by plotting all the raw scores, as shown in Fig. 1, epitomizes the skewed frequency which was typical of nearly all the individual curves. Probably the most important factor in causing this marked positive skew was the length of the lancet blade. Fig. 1, showing the curve of the means of 10 punctures, illustrates the fairly normal distribution characteristic also of the other means and medians.

To eliminate all errors arising from the asymmetrical distribution, the data in Fig. 1 were also analyzed in terms of per cent, using the means of 10 as the point of reference. The 128 cases were divided into two groups, those having a mean bleeding time (mean of 10 punctures) of one minute or less and those

having a mean bleeding time between one and two minutes. The per cent of these groups with a bleeding time of one, two, or three minutes on the basis of the other means and medians is shown in Table IV.

TABLE IV

COMPARISON OF SHORTER TESTS WITH THE MEAN BLEEDING TIME OF 10 PUNCTURES

MEASURE OF BLEEDING TIME	A % OF PERSONS WHOSE BLEEDING TIME IS 0-60" (MEAN OF 10 PUNC- TURES) HAVING B. T. 1', 2', OR 3'			SCORE*	B % OF PERSONS WHOSE BLEEDING IS 61-120" (MEAN OF 10 PUNC- TURES) HAVING B. T. 1', 2', OR 3'		
	1'	2'	3'		1'	2'	3'
Mean of first 6 made	91	9	0	169	16	78	6
Mean of first 6 that bled	78	22	0	160	10	82	8
Mean of first 4 made	86	14	0	150	32	64	4
Mean of first 4 that bled	76	24	0	150	20	74	6
Median of first 4 made	88	12	0	144	36	56	8
Median of first 4 that bled	79	21	0	145	26	66	8
Median of first 3 made	93	7	0	145	44	52	4
Median of first 3 that bled	85	15	0	147	32	62	6
Mean of first 3 odd that bled	86	14	0	152	31	66	3

*This score is the sum of the per cent values in column 1A and column 2B. It is an arbitrary measure for evaluation.

The measures which included zero readings show the greatest per cent of agreement with the group which had a bleeding time of one minute or less (on the basis of the mean of 10), and the measures excluding the zeros were in better agreement with the group having a bleeding time between one and two minutes. By summing the per cent values listed for each test in column one (1') of the first group with those of column two (2') of the second group, an arbitrary score was devised by which to evaluate the tests. As was pointed out in the discussion of the correlations of the means, the inclusion of the same raw data in computing the two measures, gives an advantage to those based on the largest number of punctures. As in the previous case an exception appears in favor of the mean of the first three odd punctures that bled.

TABLE V

BLEEDING TIME AVERAGES OF THE SUCCESSIVE PUNCTURES

SUBJECTS	1	2	3	4	5	6	7	8	9	10	TOTAL
All cases	47.4	53.7	55.6	64.9	53.0	76.9	59.4	67.9	62.5	71.2	61.5
All cases—zeros excluded	59.5	66.1	61.4	72.3	59.6	85.6	64.6	73.6	66.6	75.3	68.4
Men only	43.4	56.7	60.6	63.3	55.9	77.0	62.0	70.8	70.6	72.5	63.2
Women only	48.4	50.9	51.0	67.6	50.4	76.7	57.0	65.2	55.1	70.0	59.2

The fourth consideration in selecting one of the proposed bleeding time measures is the obvious advantage of brevity. The assumption that the test would be improved by increasing the number of punctures was invalid in this case. As is shown in Table V there is a decided trend toward a longer bleeding time as the test proceeded. This is also shown by the fact that the mean of the first 5 punctures (128 cases) is 54.38, and the mean of the last 5 is 68.59. The

difference between the two means is statistically significant, since the critical ratio is 5.24. *A progressive hyperemia seems to be the most pertinent interpretation of this cumulative error.*

Because the first two punctures included more than a third of the zero scores, and the last two were unduly prolonged, the means of the middle six punctures were computed (see Table I) and used as a standard in place of the means of 10. The substituted values did not gain sufficiently in uniformity to offset the statistical disadvantage of omitting two-fifths of the readings. This standard was, therefore, less satisfactory than the means of 10.

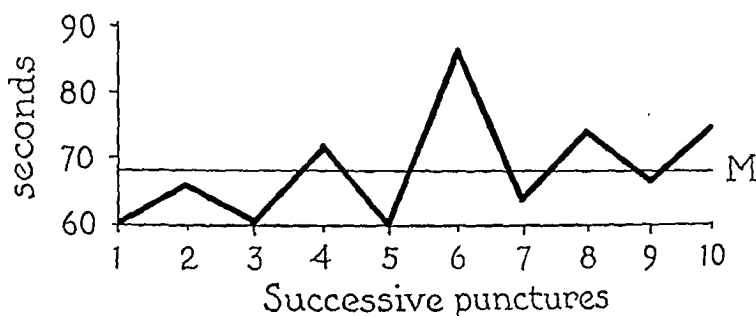


Fig. 2.—Average bleeding times of successive punctures.
Abscissae = Successive punctures.
Ordinates = Mean bleeding time in seconds. Zero readings excluded.
M = Mean of all punctures. Zero readings excluded.

Does a first puncture modify the bleeding of a second puncture made five seconds later? Table V and Fig. 2 also reveal another source of error, where one puncture follows another in five seconds. Apparently the first puncture of each pair was sufficiently traumatic to cause a transitory hyperemia or change, probably due to an axone reflex. The only exception to the sharp alternations is puncture 2, when the zero readings are included. When all the first punctures, the odd tests, are compared with the second, or even tests, there is a significant difference between them. The mean of the odds is 56.88, the mean of the evens is 68.12, and the critical ratio is 5.70.

It is very interesting that in spite of the marked difference between them, the odd and even punctures have a correlation coefficient of +0.63. A correlation of this type constitutes the "split-half" technique for determining the reliability of a test. Considering the similarity of the data, this is a fairly high correlation, certainly far superior to the "test-retest" relationship. The fact that a correlation of single punctures made within five seconds of each other is twice as high as the correlation of the means of 10 punctures made on different days may be interpreted in two ways. It may mean that the control of all variables, except the site of puncture, as was the case with the simultaneous readings, minimized certain physiologic fluctuations; on the other hand, it may simply indicate that sufficient errors arise in the long test to impair its reliability.

The reason for making the readings in pairs was to save time. *Analysis of the results shows clearly that more uniform results are to be expected from making the punctures one at a time.*

Inspection of Fig. 2 suggests the use of the first three odd punctures that bled as the most consistent test of bleeding time. This measure compared favor-

ably with the others: (1) the 128 means thus obtained were almost identical to those which resulted from averaging 10 punctures; (2) reliability judged by the "test-retest" method was about average; (3) reliability based on deviations was relatively high; and (4) a three-puncture test is undeniably short. It seems, therefore, to fulfill all the requirements stipulated for the evaluation of the proposed bleeding time measures.

Recommendation.—Using the lancet we employed, it is recommended that a relatively reliable mean venostasis bleeding time may be determined as follows: (1) The pressure in the cuff is elevated to 40 mm. Hg. The puncture is made. If bleeding occurs, the bleeding time is recorded; if it does not, nothing is recorded. The cuff is decompressed. (2) Five minutes later the cuff is inflated again, and the puncture is made. If bleeding occurs, the bleeding time is recorded; if it does not, nothing is recorded. This procedure is repeated until three bleeding times have been recorded. The mean bleeding time is the average of the three bleeding times. In our 88 subjects the mean bleeding time of three punctures was 61.41 ± 2.43 , as compared to the mean of 10 punctures which was 61.56 ± 2.08 . It should, however, be recalled that our three punctures were the first punctures of each of three pairs and were not made singly as recommended above.

SUMMARY

Mechanical refinements directed toward increasing the accuracy of the use of a spring, flat-bladed lancet in the hands of an operator of some experience did not prove to be of significant value. The blade of the lancet was set at 3 mm. Venostasis of the forearm was produced by placing a sphygmomanometer cuff about the arm and inflating it to 40 mm. Hg pressure. The skin over the pronator was punctured with the lancet. The blood was gently blotted at ten-second intervals until bleeding ceased.

A study of the reliability of the venostasis bleeding time technique was made on 88 "normal" subjects by a multiple skin puncture method. Five pairs of punctures of the skin were made on each, the second puncture of the pair being made five seconds after the first. The cuff was deflated and the arm was moved about to restore circulation between each pair of tests. The subjects included 58 men and 30 women from about 21 to 40 years of age.

A "clinically normal" maximum venostasis bleeding time of 240 seconds for a "one puncture test" was confirmed. A "clinically normal" maximum mean venostasis bleeding time of 125 seconds was found on making 10 punctures in each of the 88 subjects. A "clinically normal" mean venostasis bleeding time of 61.56 ± 2.08 seconds was obtained for this group of 88 subjects. No significant sex difference in the mean bleeding time was found. The results on 31 subjects who were retested showed no significant variation in the mean bleeding time.

Statistical analysis of the results showed (a) that the second puncture of each pair bled significantly longer on the average than the first, (b) that the mean bleeding time of the last 5 punctures was significantly greater than the first 5, and (c) that a relatively reliable mean bleeding time for one setting may be

obtained by making one puncture during each inflation of the cuff, and averaging the time of the first three that bleed.

REFERENCES

1. Ivy, A. C., Shapiro, P. F., and Melnick, P.: The Bleeding Tendency in Jaundice, Surg., Gynec. & Obst. 60: 781, 1935.
2. McNeely, R. W., Shapiro, P. F., and Melnick, P.: The Effect of Viosterol in Jaundice, Surg., Gynec. & Obst. 60: 785, 1935.
3. Tocantins, L. M.: The Bleeding Time, Am. J. Clin. Path. 6: 160, 1936.
4. Guilford, J. P.: Psychometric Methods, New York, 1936, McGraw-Hill Book Co., 549.
5. Ibid. p. 51.

CHEMICAL

HEMOGLOBIN ESTIMATION WITH UNDILUTED REDUCED BLOOD*

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SAHLI¹ in 1902 first used the color intensity of acid hematin as a basis for the clinical estimation of hemoglobin. The original Sahli instrument has seen many modifications and reconstructions, e.g., the Anteuirith-Königsberger hemometer,² the Newcomer hemometer,³ and the Haden-Hausser instrument⁴ have been introduced. In Europe there has been constructed in recent years the Zeiss-Ikon hemometer⁵ in addition. All these models, like the original Sahli instrument, use the brown color of acid hematin which has to be compared with a standard color (fluid or glass). This "Sahli principle" is probably the one most commonly used for hemoglobin estimation in routine work. It is well known that the brown color which develops from addition of acid to the blood depends on various reactions. They in turn are supposed, as a whole, to depend on the hemoglobin content and hence, if standard conditions are used, to lead to a color intensity which is proportional to the hemoglobin previously present in the blood.

Newcomer³ in 1930 published a very profound investigation about the optical behavior of acid hematin. His experience suggested that the curve of color development against time had a rather regular slope. The question remained whether the blood solution after being acidified really "acts for the present purpose as if it formed a true solution" (Newcomer³). In a series of publications⁶⁻¹¹ during the last few years, I was able to demonstrate that the colloidal character of acid hematin solutions can very easily be evidenced by the influence upon the hemometric readings of different experimental conditions. In experiments with human blood I could show deviations as high as 14 per cent in the hematin color intensity as compared with the photometrically determined oxyhemoglobin value. I wrote (1937) with reference to the colorimetry of the hematin color, "the latter is a term of very doubtful value. It should disappear from the clinic as a measure of the hemoglobin content."¹⁰ Heilmeyer and von Mutius¹² (1938), in comparing the hematin method with other more reliable methods of hemoglobin determination, also found an inaccuracy up to 11 per cent and so did Humperdineck.¹³ Heilmeyer and von Mutius state "that the method should be eliminated as far as possible for scientific purposes"¹²; they are of the opinion that the procedure might still be satisfactory for medical practice because of the lack of any other reliable and handy method.

In 1937⁹ I pointed out that the colorimetry of reduced hemoglobin, introduced by Bürker,¹⁴ and in connection with the use of the Pulfrich photometer recommended by Heilmeyer,¹⁵ could well be simplified with a handy instrument

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for routine work. In 1938 I learned that such an instrument had been proposed independently by the Danish physician Dr. M. Philipsen and was just brought on the market.¹⁶ After having carried out comparative investigations with the new instrument (Sieca hemometer*), a brief report in connection with my previous publications in that field may be justified.

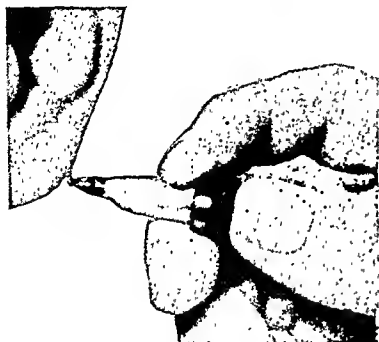
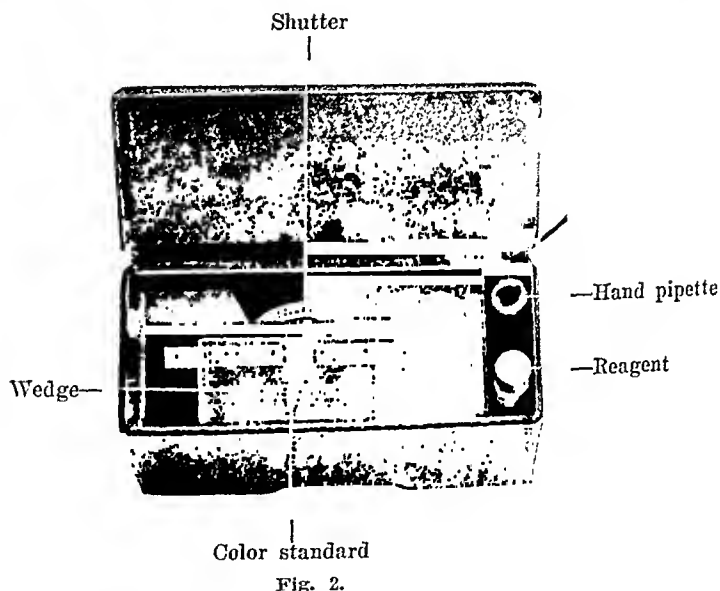


Fig. 1A.



Fig. 1B.



SICCA HEMOMETER

One of the advantages of the new method is that the blood need not be measured but simply taken in the hand pipette (Fig. 1A). Without being diluted it is reduced, hemolyzed, and stabilized by addition of a trace of a reagent powder containing sodium hydrosulfite, saponin, oxalate, and sodium chloride (Fig. 1B).

The main part of the hemoglobinometer is the wedge-shaped chamber. It is formed by a glass base plate and a covering glass piece ground to form a wedge-shaped hollow. The reduced blood specimen in the hand pipette is directed into the wedge-shaped chamber, thus forming a blood color wedge.

*Manufactured by the Testa-Laboratorium, Vedbaek, Denmark, U. S. A. Pat. No. 2,163,467.

The wedge chamber lies on the scale plate which has a window showing the standard color. Both blood and standard are illuminated from beneath by an electric lamp, the light passing some particularly selected filters. Fig. 2 shows the pattern of the apparatus. For determining the hemoglobin content the chamber is moved along until the specimen color corresponds to the standard. A marked line on the glass base plate indicates the hemoglobin value on the scale. The instrument is adapted to two ranges of hemoglobin content. This involves the use of two color standards, one of which is kept covered by a shutter. The standard most appropriate is uncovered by turning the wheel marked with an arrow. The arrow automatically points to that side of the scale to be read. An additional wedge ground to double height and marked "2" may conveniently be used for low hemoglobin values. When using this wedge all readings have to be divided by two.

TABLE I

STANDARDIZATION OF SICCA HEMOMETER WITH PHOTOELECTRIC OXYHEMOGLOBIN DETERMINATIONS

(Comparison With Hemoglobin Values Obtained From Blood Iron)

NUMBER	SICCA READINGS (MEAN OF 5-10) SCALE PARTS	HEMOGLOBIN (FROM SICCA) GM./100 C.C.	O ₂ HB. (PHOTOELECTR. EVELYN) GM./100 C.C.	HEMOGLOBIN (FROM BLOOD IRON WONG-WALKER) GM./100 C.C.
1	87.0	12.7	12.8	13.4
2	74.3	10.9	11.5	11.3
3	101.6	14.9	15.2	15.2
4	87.0	12.7	12.1	12.8
5	104.0	15.2	14.8	14.8
6	65.0	9.5	9.4	9.5
7	102.7	15.0	15.1	15.1
8	81.4	11.9	12.4	11.9
9	103.5	15.1	14.5	14.2
10	100.0	14.6	14.0	13.7
11	97.9	14.2	14.0	14.9
12	100.8	14.8	14.6	14.9
13	81.7	12.0	12.1	11.6
14	94.3	13.8	14.3	13.7
15	98.6	14.4	14.2	13.7
16	88.9	13.0	13.5	13.2
17	75.1	11.0	10.4	10.7
18	85.9	12.6	12.5	13.1
19	111.7	16.4	16.7	16.4
20	87.9	12.9	13.7	13.1
21	100.9	14.8	15.3	14.0
22	91.2	13.3	12.7	13.1
23	90.0	13.2	13.2	13.2
24	82.3	12.0	12.1	12.5
25	108.1	15.8	16.0	16.1
Average	92.07		13.48	13.44

Therefore 100 scale parts Sicca = 14.64 grams of hemoglobin per 100 c.c.

STANDARDIZATION

Because of a mistake in shipping, I had to build in a new set of filters in the instrument I used first. Thus a restandardization of the hemometer became necessary. Twenty-five samples were taken from different patients and protected against coagulation by adding some oxalate or liquid "Roche." The hemoglobin in each sample was determined electrophotometrically according to Evelyn.¹⁷ In addition the iron content of the blood was determined following

Walker's¹⁸ modification of Wong's method. The hemoglobin was calculated on the basis of 0.336 per cent iron. The same 25 samples were also used for hemoglobin determinations in the Sica hemometer. The results of five to ten readings with each sample were averaged. Table I contains the synopsis of the results.

The average of the readings on the scale in the 25 blood samples was 92.07. The average for the oxyhemoglobin content photoelectrometrically measured, according to Evelyn, was 13.48 Gm. per 100 c.c. From these two figures was calculated that 100 parts on the scale of this particular instrument (A) equal 14.64 Gm. of hemoglobin per 100 c.c. Using this correlation, the hemoglobin content for each of the 25 samples was calculated from the respective Sica readings. The results are shown in Table I and can be compared with the respective figures from the photoelectrical readings and the iron determinations.

TABLE II

COMPARISON BETWEEN TWO SICCA HEMOMETERS (A AND B)
A Standardized by the Author; B Standardized by the Manufacturers

NO.	GRAMS HB. PER 100 C.C.		NO.	GRAMS HB. PER 100 C.C.	
	A	B		A	B
1	12.5	12.4	13	14.3	14.0
2	12.6	12.8	14	12.9	13.2
3	14.6	15.0	15	11.9	11.7
4	14.9	15.2	16	11.9	12.3
5	8.7	8.7	17	13.3	13.4
6	12.3	12.2	18	13.1	13.1
7	13.1	13.1	19	12.6	12.4
8	12.7	12.7	20	12.0	11.9
9	13.3	13.5	21	13.2	13.2
10	12.4	12.5	22	13.9	14.2
11	15.6	16.2	23	13.8	13.6
12	12.8	12.9	24	14.5	14.4
			25	14.6	14.4

TESTING OF MANUFACTURERS' STANDARDIZATION

The second Sica hemometer (B) was standardized by the manufacturers in Denmark in the manner commonly used there (in cooperation with Professor A. Krogh's Zoofysiologisk Laboratorium) on the basis of 100 parts of the scale equal 18.5 volume per cent of oxygen (Haldane); 18.5 volumes per cent of oxygen correspond to 13.85 Gm. of hemoglobin per 100 c.c. Thus the latter is at the same time the standardized hemoglobin value for 100 parts on the scale. In other words, one part on the scale means a little less hemoglobin with the instrument B than with the instrument A. A total of 25 other human blood samples were read in the two Sica hemometers. The same wedge chamber filled with the prepared blood was placed first in one instrument, and after the readings were taken (usually 5 to 10) placed in the other one. The order of the use of the two hemometers was alternating, thus also controlling any influence of time. The readings for each instrument were multiplied by their respective factors (0.1464 for A and 0.1385 for B). Table II shows the results obtained.

The hemoglobin content of the samples varied from about 8.7 to about 16.0 grams per cubic centimeter. The average of the values for the 25 blood samples

obtained with the two instruments is 13.1 and 13.2 Gm. per 100 c.c. In the individual results there is also a good agreement between the two instruments. The deviation is less than 1 per cent in 19 of the 25 cases, and never exceeds 2 per cent of the average reading.

The low dispersion for individual readings was also proved in the following manner: In 50 determinations with the Sieca hemometer in which ten readings had been taken for each sample, the average of the first three (R_3) and of the ten readings (R_{10}) was compared. In 48 of the 50 cases the per cent deviation of the two averages: $\frac{\pm (R_3 - R_{10})}{R_{10}} \cdot 100$ was not greater than 2; in the remaining 2 cases the deviation was 2.5 and 3.5 per cent, respectively. This means that by taking the average of three readings with the same specimen one can be certain of having a result correct for all practical purposes.

INTERCHANGEABILITY OF PARTS

One of the most important claims for the Sieca hemometer is that all spare parts are supposed to be interchangeable without restandardization. The parts of the wedge chamber evidently are the most vulnerable elements of the instrument in practical use. Three different base plates equipped with three different wedges were filled with blood from one sample. The readings were taken in the same instrument. The hemoglobin values obtained for the three wedge chambers were: 10.54; 10.64, and 10.45 Gm. per 100 c.c., respectively. This means a deviation of less than 1 per cent from the average.

Table III gives the average results of low hemoglobin values in four cases, two of them artificial dilutions, and two cases of pernicious anemia. The data obtained with wedge "2" in the Sieca are compared with those obtained with Evelyn's photoelectric method.

TABLE III

LOW HEMOGLOBIN VALUES DETERMINED WITH SIECA AND PHOTOELECTRICALLY

SAMPLE	SIECA HEMOMETER WEDGE "2" GM. HB./100 C.C.	PHOTOELECTRIC METHOD EVELYN GM. HB./100 C.C.
Diluted blood I + 0.9 per cent NaCl	5.33	5.41
Diluted blood II + 0.9 per cent NaCl	3.84	3.92
Pernicious anemia; Case P.	5.33	5.30
Pernicious anemia; Case C.	5.36	5.44

The photoelectric oxyhemoglobin determination (Evelyn) and the results obtained with the Sieca instrument, as a rule, were in very close agreement for the low as well as for the normal hemoglobin concentrations. In this connection it is of particular interest that, according to personal information by Dr. H. Hesse, a large series of hemoglobin estimations carried out in Montreal, Canada, with the Sieca instrument and with Van Slyke's method of determination the oxygen capacity never showed deviation exceeding 3 per cent.

SUMMARY

In my opinion some advantages of the new type of hemoglobinometer are:

(1) The use of undiluted blood eliminates the sources of error involved in diluting. The handling of the Sieca hemometer is very simple and needs no particular training.

(2) The use for colorimetry of acid hematin, repeatedly criticized in earlier work, is abandoned in favor of the color of reduced blood. The latter is stable and independent of time, the errors due to inadequate time for reading being eliminated.

(3) The use of constant glass standard and constant electric light in connection with correct filters allows observations with great optical contrast, eliminates the effects of voltage variations and plasma color (icteric blood), and decreases the personal equation to a minimum.

(4) The dispersion of repeated readings with the same or with different Sieca hemometers is small, i.e., the reproducibility is particularly good.

(5) Spare parts can be used without new standardization.

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REFERENCES

1. Sahli, H.: Über ein einfaches und exactes Verfahren der klinischen Hämometrie, Verhandlungen des Congresses für innere Medizin 20: 230, 1902.
2. Autenrieth, W., and Koenigsberger, J.: Über ein neues Kolorimeter und dessen Verwendung zur Bestimmung von Blutfarbstoff, Eisen, Indikan, und Kreatinin, München. med. Wchnschr. 57: 998, 1910.
3. Newcomer, H. S.: Absorption Spectra of Acid Hematin, Oxyhemoglobin and Carbon Monoxide Hemoglobin; A New Hemoglobinometer, J. Biol. Chem. 37: 465, 1919.
4. Haden, R. L.: A New Hemoglobinometer, J. LAB. & CLIN. MED. 16: 68, 1930. A Clinical Model of the Haden-Hausser Hemoglobinometer, J. LAB. & CLIN. MED. 18: 1062, 1933.
5. Rostoski: Ein neues Hämoglobinometer, Verhandl. d. deutsch. Gesellsch. f. inn. Med. 46: 365, 1934.
6. Barkan, G.: Zur Frage des Blutfarbstoffzerfalls durch verdünnte Säuren, Biochem. Ztschr. 224: 53, 1930.
7. Barkan, G.: Über das optische Verhalten von O₂-Hämoglobin- und CO-Hämoglobin-Blutlösungen nach Säurezusatz, Biochem. Ztschr. 283: 241, 1936.
8. Barkan, G., and Olesk, J.: Zur Bewertung der üblichen Hämoglobinbestimmungen, Klin. Wchnschr. 15: 454, 1936.
9. Barkan, G., and Olesk, J. (in collaboration with Kurvits-Sehnee, H.): Über den Farbwert surer Hämatinlösungen, Biochem. Ztschr. 289: 251, 1937.
10. Barkan, G.: Die optische Bestimmung des Blutfarbstoffs als Oxyhämoglobin, Hämoglobin und Hämatin und die Zuverlässigkeit der Ergebnisse, Biochem. Ztschr. 294: 239, 1937.
11. Barkan, G.: Hämoglobinbestimmung, Hämometereichung und die Veröffentlichung optischer Konstanten, Deutsches Arch. f. klin. Med. 184: 114, 1939.
12. Heilmeyer, L., and von Mutius, I.: Über die optische Bestimmung des Hämoglobins als Oxyhämoglobin, reduziertes Hämoglobin und Hämatin, Deutsches Arch. f. klin. Med. 182: 164, 1938.
13. Humperdinck, K.: Zur Frage der colorimetrischen Bestimmung des Hämoglobins als reduziertes Hämoglobin oder als salzsaures Hämatin auf Grund zahlreicher gewerbeärztlicher Blutuntersuchungen, Deutsches Arch. f. klin. Med. 183: 379, 1939.
14. Bürker, K.: Genauere Hämoglobinbestimmungen und Erythrocytenzählungen, Abderhalden's Handb. d. biol. Arbeitsmeth. IV, 4: 1203, 1927.
15. Heilmeyer, L.: Medizinische Spektrophotometrie, Jena, 1933, G. Fischer, p. 86.
16. Hesse, H., and Trier, M.: Haemoglobinbestemmelse paa ufortyndet Blod, Ugeskr. f. laeger 99: 935, 1937; Poulsen, J.: Haemometri paa ufortyndet Kapillaer-blood paa Hospitaler og i Praksis, Ugeskr. laeger 100: 224, 1938; Hesse, H.: Hämoglobinbestimmung mit unverdünntem Blut, Acta med. Scandinav. 97: 207, 1938; cf. also Lane, C. R.: A Rapid and Accurate Method of Hemoglobin Determination, Brit. M. J. Jan. 23, p. 167, 1939, and Philipsen, M.: Behøver Laegen et nøjagtigt Haemometer i den daglige Praksis? Ugeskr. f. laeger 101: 415, 1939.
17. Evelyn, K. A.: A Stabilized Photoelectric Colorimeter With Light Filters, J. Biol. Chem. 115: 63, 1936.
18. Walker, B. S., and Fith, R.: Clinical Observations on Blood Iron, Ann. Int. Med. 14: 263, 1940.

THE MEASUREMENT OF CELL VOLUME OF BLOOD BY THE EVANS BLUE DYE METHOD*

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THE hematocrit method for the determination of the cell volume in blood, as developed by Wintrobe,¹ Guest,² and others, has been of great service in both physiologic and clinical studies. It has the advantage of being simple and direct, and further, it gives separate readings for erythrocytes and leucocytes. It requires approximately 1 c.c. of blood. Careful studies of this method by Ponder³ and Hirota⁴ have shown, however, that it is not without its difficulties. The values obtained at 3,000 r.p.m. for thirty minutes, which is the condition commonly used, are arbitrary values, but, Ponder states, are correct ± 5 per cent. It is generally accepted that the error is within 2 or 3 per cent. Low speed and short duration of centrifugation result in a large volume of packed cells. Centrifugation at higher speeds or for longer times causes the volume of packed cells to become smaller to the point where fluid is squeezed out of the cells by centrifugal force. Moreover, various bloods do not attain the same equilibrium at the same time or speed of centrifugation.

A method for determination of cell volume, called the cell opacity method, has recently been described.⁵ This method depends upon the obstruction to light, of 660 $m\mu$, by a suspension of blood in citrate solution. The amount of light transmitted actuates a photoelectric cell, and the intensity of the current is measured by a galvanometer in a photoelectric colorimeter. An empirical correlation between this reading and the cell volume obtained by the hematocrit method is made. The method uses only 10 or 20 c. mm. of blood and requires only a few seconds to carry out. The values on relatively normal bloods agree within 2 or 3 per cent with those obtained by the hematocrit method.

To obviate the difficulties of the hematocrit method and to serve as a standard of reference, Ponder⁶ suggested the use of a colorimetric method based upon the addition of a solution of hemoglobin in plasma to whole blood from the same individual. The hemoglobin concentration of the plasma is determined before and after the addition, and the volume of the cells is thus calculated. The technical difficulties of this method in our hands have been considerable. It is open to the further objection that about 35 c.c. of blood are required for each determination. Further, what Ponder calls a solution of hemoglobin really contains all the soluble parts of the cell, high in potassium and

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phosphate, obtained by freezing and lysis, and perhaps some cellular detritus. Practically, the effect of these substances on osmotic pressure is greatly reduced because the hemoglobin solution is diluted 1:15.

The method reported here is a colorimetric method which we think is free from these objections. The principle of the method is as follows: A known amount of blood is mixed with a given amount of the dye, Evans blue, also called T-1824. The concentration of the dye in the plasma is determined colorimetrically with a photoelectric colorimeter. Because the dye is contained wholly within the plasma, the volume of the plasma is determined by this measurement; from this the relative amount of the plasma in whole blood is calculated as per cent, and the cell volume is obtained by difference. The method is more laborious than the hematocrit method or the cell opacity method. It is offered primarily as a standard of reference rather than as a clinical method, although it may be so used.

PROCEDURE

Evans blue (Merek) is dried at 110° C. and made up into solution with distilled water so that it contains 400 to 800 mg. of dye per liter. One half to 1 c.c. of this solution (containing 400 μ g. of dye) is placed in a test tube of 3 to 4 c.c. capacity and evaporated to dryness at 70° C.

A measured amount of blood obtained by venepuncture is placed in a tube coated with a calculated amount of dried oxalate (60 per cent ammonium oxalate and 40 per cent potassium oxalate) or heparin to give a final concentration of 0.2 per cent oxalate or 2.0 to 2.5 units of heparin per cubic centimeter of blood. An aliquot is centrifuged. One cubic centimeter of plasma is placed in a tube containing the dye as described above (or a smaller volume but in the same proportion to the dye). The tube is shaken and the dye readily goes into solution. Of the plasma containing dye 0.1 c.c. is removed and placed in 9.9 c.c. of physiologic saline solution (0.85 per cent of sodium chloride) in a tube standardized for use in the photoelectric colorimeter. (The saline prevents precipitation of protein which would occur if the plasma were diluted with water.) Of the dye-free plasma 0.1 c.c. is placed in a similar colorimeter tube containing 9.9 c.c. of saline solution to serve as a "blank." The pipettes to be used for this purpose are those described by Levy.⁷ These pipettes are calibrated "to contain," and automatically deliver the required volume, because they are constricted at the upper mark to a hairline diameter. The sample of plasma is delivered into the pipette washed with the saline solution.

A suitable photoelectric colorimeter, such as the Evelyn or the Klett-Summerson colorimeter, is arranged for use with a filter transmitting light at approximately 620 $m\mu$. This filter was selected as most suitable by Gibson and Evelyn¹¹ after a study of the absorption curve of the dye. The blank is inserted and the machine is adjusted to read at 100 on the Evelyn or at 0 on the Klett-Summerson colorimeter. The dye tube is then inserted and the reading made. The dye has a very intense color, and the optimal concentrations for accurate reading in the Evelyn or Klett-Summerson colorimeter are approximately 15 to 50 μ g. of Evans blue in a volume of 10 c.c. By adding varying amounts of

saline or plasma to the dye different readings are obtained which establish a straight line, as shown in Fig. 1.

The procedure for analysis of blood is similar to the quantitation of the dye in plasma. One, 2, or 3 c.e. of oxalated or heparinized blood are placed in a tube containing 400 μ g. of Evans blue. (If 1 c.e. of blood is used, either half the amount of dye or twice the amount of diluent should be used.) The tube is stoppered and shaken until the dye is completely dissolved. The tube is then centrifuged at moderate speed, 1,500 r.p.m., for fifteen minutes. An aliquot of 0.1 c.e. of the supernatant plasma is added to 9.9 c.e. of saline in a colorimeter tube. The same plasma blank as above is used to determine the point of zero concentration of dye. The concentration of dye in the sample analyzed is obtained from the galvanometer reading by the use of the curve in Fig. 1. The cell volume is then calculated as follows:

A = amount of dye used; μ g.

Z = concentration of dye determined in supernatant plasma;
 μ g. per cubic centimeter.

S = volume of blood used; c.e.

P.V. = plasma volume; per cent of whole blood.

C.V. = cell volume; per cent of whole blood.

$$\frac{A}{Z \times S} \times 100 = \text{P.V.}; 100 - \text{P.V.} = \text{C.V.}$$

Example: A = 400; Z = 370; S = 2.

$$\frac{400}{370 \times 2} \times 100 = 54.0 = \text{P.V.}; 100 - 54.0 = 46.0 = \text{C.V.}$$

It is obvious that this method gives a measure of the volume of plasma in a given amount of blood, and hence the cell volume obtained by subtraction includes all the fixed elements in the blood, and not in the erythrocytes alone.

The first step in applying this method is for the operator to determine that he can make adequate recovery of the dye in water or saline, and then in plasma, and thus establish his curve. This curve can be used without making a plasma determination for each blood analyzed so long as the same lot of dye is used.

No attempt was made to handle the blood without loss of carbon dioxide or exposure to the air, but it was treated with reasonable care. It was kept in tubes nearly filled and always stoppered, and was analyzed within one hour after venepuncture. All the glassware was carefully calibrated. It was washed in cleaning solution before each use, and then repeatedly washed with distilled water. If the precaution of using cleaning solution is not observed, the blue dye will adhere to the glass.

Five cubic centimeters of blood are adequate for this determination in duplicate; two 1.0 c.e. samples are measured into tubes containing dye, the remainder is centrifuged to obtain plasma for the blank and for the determination of the concentration of dye in the plasma to check against the curve. If the centrifuging is done in a hematocrit tube, the cell volume may be read before the plasma is removed, and thus a comparison made without extra blood being required.

EXPERIMENTAL RESULTS

We wish to compare the results of simultaneous determinations of cell volume on the same blood by the hematocrit method, the cell opacity method, and the colorimetric Evans blue method. We used 15 c.c. of blood to enable us to make observations on known concentrations of dye in plasma on each blood, and quadruplicate determinations of cell volume employing aliquots of 1, 2, or 3 c.c. of blood. The results of the 16 consecutive determinations most recently made are given in Table I.

TABLE I

IDENTIFICATION			CELL VOLUME				COMPARISON OF RESULTS	
DATE	SUBJECT	ANTICOAGULANT	1	2	3	4	5	6
			HEMATO-CRIT METHOD R.B.C. (%)	CELL OPACITY METHOD (%)	HEMATO-CRIT METHOD R.B.C. + BUFFY COAT* (%)	EVANS BLUE METHOD (%)	1-2 (VOL./100 VOL. BLOOD)	3-4 (VOL./100 VOL. BLOOD)
12/31	T. H.	Oxalate	49.0	46.0	49.5	47.5	+3.0	+2.0
1/ 2	A. T.	Oxalate	48.0	46.5	48.5	46.2	+1.5	+1.3
1/ 2	T. H.	Oxalate	47.0	47.5	47.5	45.4	-0.5	+2.1
1/ 3	A. T.	Oxalate	47.5	45.5	48.0	47.5	+2.0	+0.5
1/ 4	T. H.	Heparin	50.0	49.5	50.5	47.5	+0.5	+3.0
1/ 6	A. T.	Heparin	47.0	48.0	47.5	45.6	-1.0	+1.9
1/14	G.†	Oxalate	31.0	28.5	31.5	30.0	+1.5	+1.5
1/16	A. T.	Oxalate	49.2	49.2	49.7	48.0	0.0	+1.7
1/17	H. S.	Oxalate	47.0	47.5	47.5	45.0	-0.5	+2.5
1/17	E. M.	Oxalate	41.7	41.4	42.2	41.0	+0.3	+1.2
1/23	T. H.	Oxalate	49.5	47.5	50.0	47.0	+2.0	+3.0
1/23	F. R.	Oxalate	46.0	45.5	46.5	44.3	+0.5	+2.2
1/24	M. C.	Oxalate	45.0	44.5	45.5	42.9	+0.5	+2.6
1/24	C. C.	Oxalate	46.0	45.5	46.5	44.5	+0.5	+2.0
1/27	J. Mc.	Oxalate	44.7	45.0	45.2	42.7	-0.3	+2.5
1/27	A. T.	Heparin	43.0	43.0	43.5	41.0	0.0	+2.5
Average			46.6	46.1	47.1	45.0	+0.5	+2.1

*A standard value of 0.5 volumes per 100 volumes of blood was added to the red blood cell volume.

†This case has been omitted from all averages for two reasons. First, it is a case of microcytic anemia, in which (as was stated in the original paper) the values obtained by the cell opacity method are too low. Second, because the total cells are only $\frac{2}{3}$ of the average for total cells, the difference between the hematocrit and the dye method should be only $\frac{2}{3}$ of the average difference between the two methods. These variations are shown quantitatively in this case.

In column 1 are given the values obtained by the hematocrit method for the volume of the red blood cells only. Hematocrit tubes were capped and spun at 3,200 r.p.m. for thirty minutes, usually in duplicate. The speed of centrifugation was checked frequently. The volumes were read ± 0.2 to 0.3 per cent of the whole blood. The readings were in agreement by the independent observations of the two observers.

In column 2 are given the cell volumes as measured by the cell opacity method. These values were determined from the line given in Fig. 2, which was established as follows: The average of the volumes of red blood cells only, obtained by the hematocrit method, was plotted against the arithmetical average of the galvanometer readings for this series of determinations. The galvanom-

eter reading for plasma alone (0 cell volume) is 98. The two points were connected by a straight line.

The results of the hematocrit method and the cell opacity method are compared in column 5. With the exception of the first case the volumes by the cell opacity method correlate with those of the hematocrit method well within the limits of the agreement originally claimed for the cell opacity method.

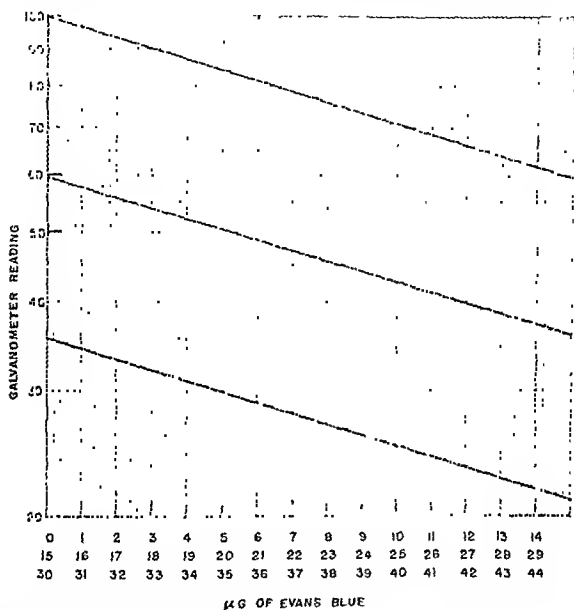


Fig. 1.—Concentration of Evans blue in plasma. This curve was made from known concentration of the dye in plasma. For each determination 0.1 c.c. of plasma was diluted to 10 c.c. with 0.85 per cent sodium chloride solution.

In column 3 are given the total cell volumes obtained by the hematocrit method. A constant value of 0.5 volumes per 100 volumes of blood was added to the red blood cell volume, as given in column 1 to obtain the total cell volume. We considered this value more accurate than actual reading of the volume of the buffy coat. We selected 0.5 rather than the more widely accepted 0.6, so that the error, if any, should make the total too small rather than too large.

In column 4 are given the cell volumes obtained by the colorimetric Evans blue method, as described under Procedure. The values were calculated from the line given in Fig. 1.

The values given in columns 3 and 4 are compared in column 6. The volumes obtained by the dye method never exceeded or even equaled those obtained by the hematocrit method, and averaged 2.1 volumes (4.5 per cent) less than the hematocrit values. Four aliquots of the same blood analyzed by the

Evans blue method have always yielded values within 1 per cent of one another (that is, 0.5 volumes of cells per 100 volumes of blood). Inasmuch as duplicate hematocrit determinations always agreed within our error of reading, and inasmuch as the absolute accuracy and causes for variation in the hematocrit method are unknown, it is impossible for us to state the difference between the two methods in accurate statistical terms. Approximate analysis shows that this value is more than five times the probable error of the difference ($\sqrt{\text{sum of the squares of the probable errors}}$) and therefore the difference is statistically highly significant. Even if the buffy coat were not taken into account the difference would still be significant.

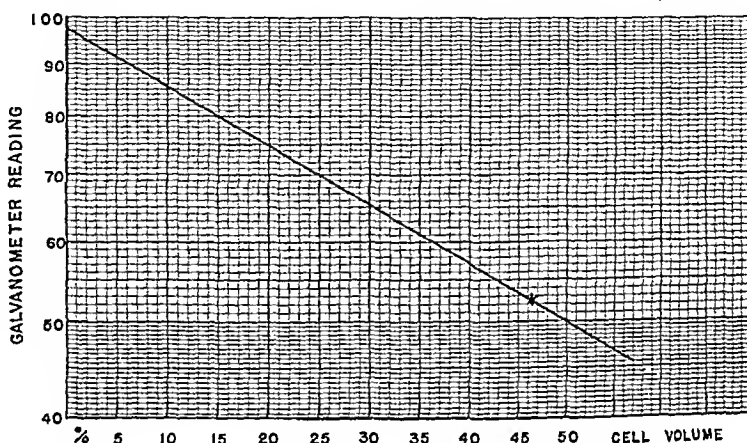


Fig. 2.—Cell volume by the cell opacity method. X = the average of the determinations in this series, which were made with 20 c.mm. of blood suspended in 10 c.c. of citrate solution and read in an Evelyn photoelectric colorimeter.

Gregersen and Schiro⁸ found a difference of 4.2 per cent between the recovery of the dye added to whole blood and that recovered in the supernatant plasma following centrifugation. This value is in excellent agreement with the difference we found between the values for cell volume obtained by the dye method and by the hematocrit method, namely, 4.5 per cent.

It seems reasonable that the lower volumes obtained by the colorimetric method represent a truer value than that obtained by the hematocrit method. The red blood cells, whether they are biconcave disks, or cup-shaped, or spheroid, could hardly be packed so tightly that there would be no plasma between them. This deduction is subject to experimental verification by seeking for dye in the packed cells, which would prove the presence of plasma between them.

We added 5 c.c. of oxalated whole blood to a tube containing 4 mg. of Evans blue. This was thoroughly agitated until the dye was completely dissolved, and 4 c.c. of the blood were transferred to a tube with a constriction slightly below the 2 c.c. level. This tube and a hematocrit tube were centrifuged at 3,200 r.p.m. for thirty minutes. The packed cells extended above the constriction. The tube was scratched with a file at this point and broken off. The sticky packed cells were then transferred by repeated washings into 15 c.c. of physiologic saline solution. This was centrifuged and 10 c.c. of the clear supernatant solution, tinged blue, were removed and measured for concentration of Evans

blue. The volume of cells removed was determined by measuring the capacity of the tube which had contained them. From the volume of cells used, the concentration of dye, and the known cell volume, it was calculated that about 2 per cent of the dye was distributed within the space occupied by the packed cells. A similar procedure was used by Gregersen and Schiro⁸ when they washed the cell residue with isotonic saline or dye-free plasma and recovered 3.9 per cent of the dye.

Under physiologic conditions leucocytes occupy such a small percentage of the total volume of cells that they may be neglected for most clinical purposes. By the colorimetric method the total plasma is determined, and hence the total cells. Whether the packed leucocytes contained plasma in the same proportion as the packed erythrocytes may under some conditions be an important problem. If pure white blood cells could be obtained readily, the problem could be attacked in the manner just described for red blood cells. This we have been unable to do, but we made orientation experiments. Forty to 45 c.c. of blood were centrifuged in two tubes specially constructed so that the white blood cells and platelets were contained in a constricted column. The buffy coat, admixed with less than one-half its volume of red blood cells, was removed and suspended in its own plasma. Aliquots of this mixture were taken for measurement of cell volume by the hematocrit, the colorimetric, and the cell opacity methods. The values obtained by the hematocrit method were 3.0 and 3.3 per cent for the erythrocytes and buffy coat, respectively, a total cell volume of 6.3 per cent. By the Evans blue method the cell volume was 5.0 to 5.5 per cent, and by the cell opacity method it was 3.5 per cent. As in the other experiments reported the cell volume determined by the dye method was less than that given by the hematocrit method. The determination is not sufficiently accurate to state whether more or less dye is included in the packed white cells and platelets than in the packed red blood cells. It is beyond doubt, however, that the white blood cells occupy volume as recorded by the blue dye method. This experiment also confirms the previous observations made upon the blood of patients with leucemia, that the cell opacity method gives values little influenced by the white blood cells.

DISCUSSION

The behavior of Evans blue dye in blood has been exhaustively studied by Gregersen, Gibson, and their collaborators,⁸⁻¹¹ who have used it successfully in the determination of plasma volume in vivo. These investigators were so interested in the use of the dye for this purpose that they did not point out the applicability of their findings to the determination of the relative volumes of plasma and cells in whole blood.

We have made the assumption that Evans blue is contained wholly in the plasma and does not enter the cells. Gregersen and Schiro⁸ concluded that the dye is dissolved only in the plasma, combined with the serum protein, and that the red blood cells do not permit either penetration or absorption of the dye. In agreement with their observations we found, as detailed above, that dye contained in packed cells is readily transferred to saline solution. Further, although we used 1, 2, and 3 c.c. aliquots of blood with the same or different

amounts of dye, we have obtained the same cell volume calculated from all aliquots, within the experimental error. If the dye had been adsorbed on, or had penetrated into, the cells this finding would mean that, regardless of concentration, the same proportion of dye was held by the red blood cells, which is highly unlikely.

In determining cell volume by the hematocrit method the effort was made to have the speed of centrifugation greater than rather than less than the usual number of revolutions per minute, and thus to err on the conservative side. The larger volumes obtained by the hematocrit method cannot, therefore, be attributed to spinning the blood at too slow a speed.

The reason for making a new curve for the cell opacity method based on the present data instead of using the one previously published, was that the latter would be interpreted in terms of red blood cell volume as one or two volumes too great. The measurement of cell opacity is an independent physical measurement, and its relation to the hematocrit method is purely empirical. The absolute value for cell volume calculated from it, therefore, is theoretically not of prime importance; the main virtue of the method lies in the fact that the correlation between it and another method is close under a given set of conditions. The original data consisted of observations on a large number of children, some of which were above and some below the line adopted. In this case we have used few individuals, all adults; mostly with high cell volumes, and it may be that we have accidentally selected individuals whose cell opacity readings fell below the original line. All the methods in this study were carried out with the greatest precision. In the former study only clinical exactness was attempted. Therefore, especially for high cell volumes, this curve is more accurate. The difference between this curve and the former one decreases as the cell volume becomes smaller.

In this series of observations we have been unable to detect any consistent difference between values whether 0.2 per cent of oxalate or 1 mg. of heparin per cubic centimeter of blood was used as anticoagulant. Neither caused swelling nor shrinking of the cells sufficient to be demonstrated, even when aliquots of the same blood were compared.

Hemolysis was not visible in any samples of blood studied. If any was present, it was probably too small to affect the cell volume significantly. In any event it was the same in the aliquots compared by the three methods. In their studies of blood volume in vivo by the use of Evans blue, Gibson and Evelyn¹¹ found it necessary to analyze successive samples of blood in which the degree of hemolysis varied. They have shown how to correct adequately for this complicating factor. Such correction does not apply to the present study, for the dye-free plasma is used for the blank, and any interference due to hemolysis is thus eliminated. In Gibson and Evelyn's studies it was necessary to use undiluted plasma in order to obtain a concentration of dye sufficient to read in the colorimeter. Under our experimental conditions any amount of dye up to the saturation point may be used, thus eliminating the necessity for microtechnique. We used sufficient dye so that the color could be read to maximum

advantage when the plasma was diluted to 100 times its volume. This also reduced to a minimum any difficulties which might be due to any interfering substances in the plasma.

We are at a loss to account for the fact that Ponder's findings are not in agreement with ours concerning the relationship between colorimetric and hematocrit methods. He presented a table⁷ which he stated "is representative of the results of many experiments." In this he compared the cell volumes given by his colorimetric hemoglobin method with those obtained by centrifuging the blood at various speeds and for various times. Two samples of blood analyzed by both methods gave values for the volume of a single cell of 85 and 78 μ^3 by the hematocrit method (spun at 4,000 r.p.m.), and 83 and 82 μ^3 by the colorimetric method, respectively. Thus each method gave values both higher and lower than the other. He did not include the buffy coat.* In our experience the colorimetric hemoglobin method yielded results more variable than did the Evans blue method. However, the hemoglobin method, as well as the Evans blue method, gave a lower average for cell volume than the hematocrit method.

SUMMARY

A colorimetric method is presented for the determination of the relative volume of the plasma and cells in blood by the use of the Evans blue dye. The error is less than 1 per cent.

By this method the total cell volume averages approximately 4.5 per cent (2.1 volumes per 100 volumes of blood) less than that obtained by the hematocrit method. This discrepancy is probably accounted for by the plasma which lies between the packed cells.

REFERENCES

1. Wintrobe, M. M.: Simple and Accurate Hematocrit, *J. LAB. & CLIN. MED.* 15: 287, 1929.
2. Guest, G. M., and Siler, V. E.: Centrifuge Method for the Determination of the Volume of Cells in Blood, *J. LAB. & CLIN. MED.* 19: 757, 1934.
3. Ponder, E.: The Mammalian Red Cell and the Properties of Hemolytic Systems, Berlin, 1934, Borntraeger, Protoplasma Monographs, Vol. 6, Chapter 11.
4. Hirota, K.: *J. Biophysics*, Japan 1: 233, 1925. Cited by Butler, A. M., and MacKay, E. M.: *J. Biol. Chem.* 106: 107, 1934.
5. Shohl, A. T.: The Measurement of Cell Volume of Blood by the Cell Opacity Method, *J. LAB. & CLIN. MED.* 25: 1325, 1940.
6. Ponder, E., and Saslow, G.: The Measurement of Cell Volume, *J. Physiol.* 70: 18, 1930.
7. Levy, G.: Studies on Enzymatic Histochemistry XVII. A Micro Kjeldahl Estimation, *Compt. rend. d. trav. du lab. Carlsberg, série chimique* 21: 101, 1936.
8. Gregersen, M. I., and Schiro, H.: The Behavior of the Dye T-1824 With Respect to Its Absorption by Red Cells and Its Fate in Blood Undergoing Coagulation, *Am. J. Physiol.* 121: 284, 1938.
9. Gregersen, M. I.: Analysis of Colorimetric Methods in Relation to Plasma Volume Determinations, *J. LAB. & CLIN. MED.* 23: 423, 1938.
10. Gibson, J. G., II, and Evans, W. A.: Clinical Studies of the Blood Volume. I. Clinical Application of a Method Employing the Azo Dye "Evans Blue" and the Spectrophotometer, *J. Clin. Investigation* 16: 301, 1937.
11. Gibson, J. G., II, and Evelyn, K. A.: Clinical Studies of the Blood Volume. IV. Adaptation of the Method to the Photoelectric Microcolorimeter, *J. Clin. Investigation* 17: 153, 1938.

*Personal communication.

PLASMA ALBUMIN, GLOBULIN, AND FIBRINOGEN IN HEALTHY INDIVIDUALS FROM BIRTH TO ADULT LIFE*

I. A SYSTEM OF MICROANALYSIS

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FOR the past eight years we have been gathering data on the levels of the plasma proteins in healthy infants and children. In this work it is frequently necessary to use very small samples of plasma. To accomplish our task we have adapted existing analytical methods to microprocedures and have added certain techniques, all of which taken together have developed into a system of analysis for the plasma proteins that has become routine in our laboratory.

In view of the relative dependability of salting-out methods, we have based our separation of the plasma protein fractions on procedures similar to those of the "Howe technique."¹ With respect to the separation with sodium sulfate, we have found that this procedure may be carried out at room temperature and that, when filtration is used for the removal of the precipitated globulin, there is a loss of albumin seemingly due to adsorption by the filter paper. During the course of the present work, both of these findings have been reported by Robinson, Price, and Hogden,^{2, 3} and the latter one has been confirmed by Harris.⁴ In addition, we have found it possible to determine serum globulin, or plasma globulin plus fibrinogen, directly in samples which do not show a marked lipemia. The nitrogen content of the fractions is determined by means of a micro-Kjeldahl procedure. We have devised a distillation apparatus which may be easily and inexpensively constructed from ordinary laboratory glassware, and which has proved very satisfactory in the hands of many workers in this laboratory.

In this, as in any microanalytic work, the factor most essential to success is strict attention to detail. We are, therefore, presenting our method at some length, together with a critical analysis of the results obtained. In the near future we shall present the values obtained in our study of healthy individuals from birth to adulthood.

METHOD

A complete analysis consists of duplicate determinations of each of the following fractions: (1) total nitrogen, (2) nonprotein nitrogen, (3) fibrinogen, (4) albumin plus nonprotein nitrogen, and (5) globulin plus fibrinogen. For this, eight 0.1 c.c. samples of plasma are needed. In measuring the samples, 0.1 c.c. pipettes, which have been recalibrated, are used. The calibration is made

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on the volume each pipette will contain. Throughout the procedure, all tubes are kept tightly stoppered to avoid errors due to evaporation.

Reagents:

- 10% sodium tungstate, nitrogen free
- $\frac{2}{3}$ N sulfuric acid, nitrogen free
- Sodium hydroxide-potassium dihydrogen phosphate buffer for fibrinogen and globulin precipitants:
- 10.05 Gm. of potassium dihydrogen phosphate
- 508.0 c.c. of 0.1 N sodium hydroxide
- Water to make 1 liter

According to Howe,⁵ this amount of potassium dihydrogen phosphate per liter would be equivalent to 6.98 Gm. of sodium sulfate for the precipitation of fibrinogen, or 7.76 Gm. of sodium sulfate for the precipitation of globulin.

Fibrinogen Precipitant:

- 104.6 Gm. of sodium sulfate (anhydrous)
- Buffer to make 1 liter

This solution has an effective precipitating strength of 104.6 Gm. plus 6.98 Gm., or 111.58 Gm. of sodium sulfate per liter. After dilution with plasma (0.1 c.c. of plasma to 1.9 c.c. of precipitant), the strength is 106.0 Gm. per liter.

Globulin Precipitant:

- 218.5 Gm. of sodium sulfate (anhydrous)
- Buffer to make 1 liter

The final effective strength of the globulin precipitant after dilution with plasma and correction for the buffer salts is 215.0 Gm. per liter.

Both the fibrinogen and globulin precipitants may be slightly hazy at first, and after a few days may develop a flocculent precipitate. The solutions with the precipitates may be used, provided that 5 c.c. samples are found to be free of nitrogen after digestion, distillation, and nesslerization. Because of the buffer present, the solutions cannot be directly nesslerized.

Digestion Mixture:

- | | |
|-----------------------------------|-----------|
| Concentrated sulfuric acid (C.P.) | 3 volumes |
| "Syrupy" phosphoric acid | 1 volume |

Hydrogen Peroxide, 30 per cent solution. Should not contain more than 0.025 mg. nitrogen per cubic centimeter.

Saturated Potassium Hydroxide.

Indicator:

- 1.250 Gm. of methyl red
- 0.825 Gm. of methylene blue
- 90 per cent ethyl alcohol to make 1 liter

The acidity of the indicator must be determined by titration with N/10 sodium hydroxide, so that the proper correction may be made in the final calculation.

Standard Sodium Hydroxide. A carbonate-free 0.1 N solution of sodium hydroxide is prepared and standardized against purified potassium acid phthalate in a concentration of 2 Gm. per 100 c.c.

Standard Sulfuric Acid. This solution should be free of ammonia, and for convenience, the normality should not be greater than that of the sodium hydroxide, since 1.0 c.c. of the solution is to be titrated with sodium hydroxide from a microburette of 1.0 c.c. capacity.

Nessler's Solution.

Stock Ammonium Sulfate, 1.0 mg. nitrogen per 1 c.c. in 5 per cent sulfuric acid.

Dilute Ammonium Sulfate, 0.04 mg. nitrogen per 1 c.c.

The solution is prepared by diluting 1 c.c. of the stock solution to 25 c.c. with water and is checked by Kjeldahl analysis of 10 c.c. portions.

*Heparin.** For plasma analysis we have used heparin as the anticoagulant.

PREPARATION OF THE SAMPLES

Total Nitrogen. One-tenth cubic centimeter of plasma is added to 1.0 c.c. of water in a 25 by 200 mm. pyrex test tube. The pipette is washed several times with the mixture and allowed to drain thoroughly. The final drop is blown out. One cubic centimeter of digestion mixture is added.

Nonprotein Nitrogen. One-tenth cubic centimeter of plasma is added to exactly 1.0 c.c. of water in a 15 c.c. pointed centrifuge tube. The pipette is washed with the mixture, drained, and the last drop is blown out. One-tenth cubic centimeter each of 10 per cent sodium tungstate and $\frac{2}{3}$ N sulfuric acid are added. The mixture is stirred with a small glass rod, care being taken not to spread the precipitate over the tube above the fluid level. This is centrifuged (fifteen minutes at about 1,800 r.p.m.), and the clear fluid is transferred by means of a capillary tube to a clean dry test tube. Great care must be taken to avoid including any particles of the precipitate during this transfer. An aliquot of 0.7 c.c. of this is placed in a 25 by 200 mm. pyrex test tube, and 1.0 c.c. of digestion mixture is added.

Fibrinogen. One-tenth cubic centimeter of plasma is added to exactly 1.90 c.c. of fibrinogen precipitant in a 15 c.c. tapered centrifuge tube, the pipette is washed three times with the solution and drained. In adding the plasma, the tip of the pipette is placed about $\frac{1}{4}$ inch under the surface of the precipitant and allowed to drain. The plasma rises from the tip to float on the surface, and the pipette is carefully washed three times with the clear underlying fluid. In the final washing, the pipette, filled with clear fluid, is withdrawn so that the tip rests against the tube just above the fluid. The pipette is allowed to drain while being rotated against the tube, thus washing back any particles adherent to its outer surface. The final drop is blown from the end of the pipette, and the contents of the tube are mixed by rotation and allowed to stand. After standing for three hours, the precipitated fibrinogen is centrifuged down to a compact, cohesive mass. This requires ten to fifteen minutes at about 1,800 r.p.m. The clear supernatant fluid is poured off, and the tube is inverted over filter paper to

*Heparin, obtained from the Connaught Laboratories, Toronto, containing 110 units per milligram has been found most satisfactory.

drain. One and nine-tenths to 2.0 c.c. of fibrinogen precipitant are added to the tube, and the contents are mixed, centrifuged, decanted, and drained. The fibrinogen pellet is transferred quantitatively to a 25 by 200 mm. pyrex test tube by means of 2 to 5 c.c. of water. One cubic centimeter of digestion mixture is added.

In precipitating fibrinogen it was found that, although the protein had flocculated within three hours, longer standing tended to yield slightly higher values. A series of fibrinogen determinations were done on 83 samples of plasma in which two fractions of each sample were allowed to stand three hours, after which the supernatant fluids were removed and allowed to stand for twenty-one hours longer. Another set of duplicates was allowed to stand for twenty-four hours before being centrifuged. The mean value for those standing for three hours was 0.188 per cent fibrinogen, and for twenty-four hours, 0.204 per cent. In 8 of the plasma samples the increase after three hours was greater than 0.04 per cent, an amount easily detected; yet in no case was there any further salting-out of protein in the supernatant fluids removed after three hours, as would be expected if the increment were due to continued precipitation of the fibrinogen. Consequently, it was deemed possible that the increase with longer standing may be due to adsorption of other proteins upon the already precipitated fibrinogen. In a series of analyses of 5 samples of plasma, the fibrinogen was determined after intervals of one hour, two, three, six, nine, twelve, and twenty-four hours' standing. In each case there was a sharp rise to the three-hour sample, after which the curve leveled. It was, therefore, decided to standardize the procedure, using this interval of time for the fibrinogen precipitation.

Albumin Plus Nonprotein Nitrogen. One-tenth cubic centimeter of plasma is added to exactly 1.90 c.c. of globulin precipitant in a 15 c.c. tapered pyrex centrifuge tube. The addition is made in the same manner as that used for fibrinogen, and the contents of the tube are mixed and allowed to stand for three hours at room temperature. We have found that longer standing, up to twenty-four hours, does not alter the amount of globulin precipitated. The tubes are then centrifuged for forty-five to sixty minutes at 2,600 r.p.m. In all cases studied, except those showing a marked lipemia, this centrifugation has been sufficient to remove the globulin as a small, fairly compact mass, leaving a crystal-clear supernatant albumin solution. This clear solution is transferred by means of a capillary tube with bulb attachment to a clean, dry test tube. One cubic centimeter of the albumin solution is pipetted into a 25 by 200 mm. pyrex test tube, and to it 1 c.c. of digestion mixture is added. The precipitate is saved for globulin analysis.

In those cases in which the plasma shows an extreme lipemia (in our work, infants, bled one to two hours after feeding), it is difficult or impossible to remove the globulin by centrifugation. In a few such instances, the precipitate rose to the top on prolonged centrifugation, and the clear supernatant solution was removed for albumin determination. More often, however, the precipitate failed to separate completely, and it was necessary to remove it by filtration. A small (1 to 1.5 cm.) funnel was fitted with a 2.5 cm. filter paper (nitrogen free),*

*The filter paper must be tested for soluble nitrogen.

by moistening with 95 per cent ethyl alcohol and drying in a current of air. The funnel was then placed in a test tube supported inside a large, straight-walled desiccator containing globulin precipitant to a depth of one inch. The desiccator should be covered during filtration, allowing the air to maintain water equilibrium with the globulin precipitant and thus prevent evaporation from the protein solution. The solution should be repeatedly filtered through the same paper until the filtrate is clear. One cubic centimeter of filtrate is used for albumin analysis.

In cases where the solution is filtered through paper, a correction is necessary because of the albumin adsorbed on the paper. This correction is determined by running a series of albumin analyses in which the values obtained by filtration and centrifugation are compared. In a series of 50 such determinations in our work, the filtrate albumin was lower than that in the centrifuged supernatant solution by 4 per cent of the amount present in the latter sample. This figure would probably vary with the size and quality of filter paper used.* Robinson, Price, and Hogden³ have obviated the need for this correction by first saturating the paper with albumin, then filtering a second portion of the solution through the same paper. This method was not practical in our work, since only a small amount of plasma was available for complete analysis.

Centrifugation and filtration are carried out at room temperature since, in agreement with the work of Robinson, Price, and Hogden,² we have found no difference between these results and those obtained when the precipitation and removal of globulin are done at 37° C. Our work was done at 25° to 35° C. Although no determinations were completed at temperatures below this point, it seems likely that the results at 20° C. would be comparable, since sodium sulfate does not crystallize from the solution at this temperature.

Globulin Plus Fibrinogen. The precipitate remaining after removal of the albumin solution (by centrifugation) is washed with 2.0 c.c. of globulin precipitant. For this washing, one drop of the fluid is added, and the precipitate is first broken up and suspended in this small volume by gently tapping the tube, then the remainder of the 2.0 c.c. is added. To the mixture 0.1 c.c. of water is added. This amount of water dilutes the precipitant to approximately the same concentration of sodium sulfate which was used originally; however, its chief function is to lower slightly the specific gravity of the medium and to insure removal of the precipitate on subsequent centrifugation. With clear or slightly lipemic plasma samples the 0.1 c.c. of water is unnecessary, since the precipitate may be removed by centrifugation and the error caused by the higher concentration of sodium sulfate is negligible. With a moderate lipemia the globulin remains suspended upon centrifugation unless this water has been added to the washing fluid. The above mixture is centrifuged for forty-five minutes at 2,600 r.p.m., and the fluid is removed and discarded. The precipitate is washed a second time, centrifuged, and the washing fluid is discarded.

The precipitate of globulin and fibrinogen is now transferred to a 25 by 200 mm. pyrex test tube. Approximately 1 c.c. of water is added to the precipitate

*We have used E. and D. No. 613, smooth finish filter paper.

and allowed to stand for ten minutes or longer. This loosens the pellet so that it can be transferred as a whole to the digestion tube. The centrifuge tube is washed with three or more successive small portions of water. One cubic centimeter of digestion mixture is added to the precipitate plus washings, and the tube is heated in a boiling water bath for thirty minutes, or until it begins to show discoloration. This preliminary heating avoids excessive foaming in the subsequent digestion.

In cases where the globulin is originally removed by filtration, it is discarded and the amount present is determined by difference.

DIGESTION OF SAMPLES

Each of the tubes prepared as outlined above is treated in the following manner: Add 2 or 3 glass beads. Heat with a microburner until dense fumes of sulfur trioxide are given off. Cool. Add 2 drops of 30 per cent hydrogen peroxide, and heat as before. Cool. Add 1 drop of the hydrogen peroxide and again heat until fumes are given off. Cool, and repeat, again using 1 drop of hydrogen peroxide. Cool well and add about 10 c.c. of water.

DISTILLATION OF NITROGEN

Ammonia from each of the samples of albumin, globulin plus fibrinogen, and total nitrogen is distilled into exactly 1.0 c.c. of standardized N/10 sulfuric acid contained in a test tube of 20 to 30 c.c. capacity. The nonprotein and fibrinogen nitrogens are distilled into 1.0 c.c. of the acid in a Peebles-Lewis colorimeter tube.⁶ If the Peebles-Lewis colorimeter is not used, the ammonia can be distilled into a graduated test tube and an appropriate aliquot taken for nesslerization.

Fig. 1 is a diagram of the completely assembled distillation apparatus, which is similar to that of Pregl as modified by Goebel.⁷ The only important new feature is the reservoir (I) of redistilled water attached to tube B through a slightly constricted opening, as indicated in Fig. 1. We also use a current of air washed with 10 per cent sodium hydroxide and 10 per cent sulfuric acid rather than the steam distillation employed by Goebel. The inverted flask, III, serves as a trap and equalizes the air current through the apparatus. The entire apparatus may be easily assembled from ordinary laboratory glassware, and is mounted upon a single ring stand. Especial care should be taken to insure a close fit of the stopper *a* to the digestion tubes (IV) and to the tubes A and B. Any space which may fill with condensed steam serves potentially as a trap to retain ammonia. We have used a quality of stopper designated as "sulfur-free, translucent, floating stock," and have replaced the stopper at the first sign of spreading around tubes A and B, or of hardening of the rubber. Tube B has a 2 to 3 mm. hole in its upper surface about 2 cm. from the lower end, and just below stopper *a*. This allows a free passage of air and steam through the apparatus at the same time that condensed steam is draining back from B to the test tube IV.

The distillation procedure is as follows: Clamp the digestion tube (IV) in position. Place the receiver (V) containing 1.0 c.c. of acid in the beaker VI and put it into position as shown in Fig. 1. The beaker is half filled with cold water.

Fill the burette *II* by means of clamp *1*. Start the current of air with clamp *4* open; then by slowly closing *4*, the air is directed as a moderately rapid stream through the apparatus. With the air flowing, slowly add 4.0 c.c. of the saturated potassium hydroxide to tube *IV* by opening clamp *2*. After about one minute begin heating tube *IV* with a microburner, and continue heating and aeration until steam begins to enter the receiver fluid as determined by the crackling

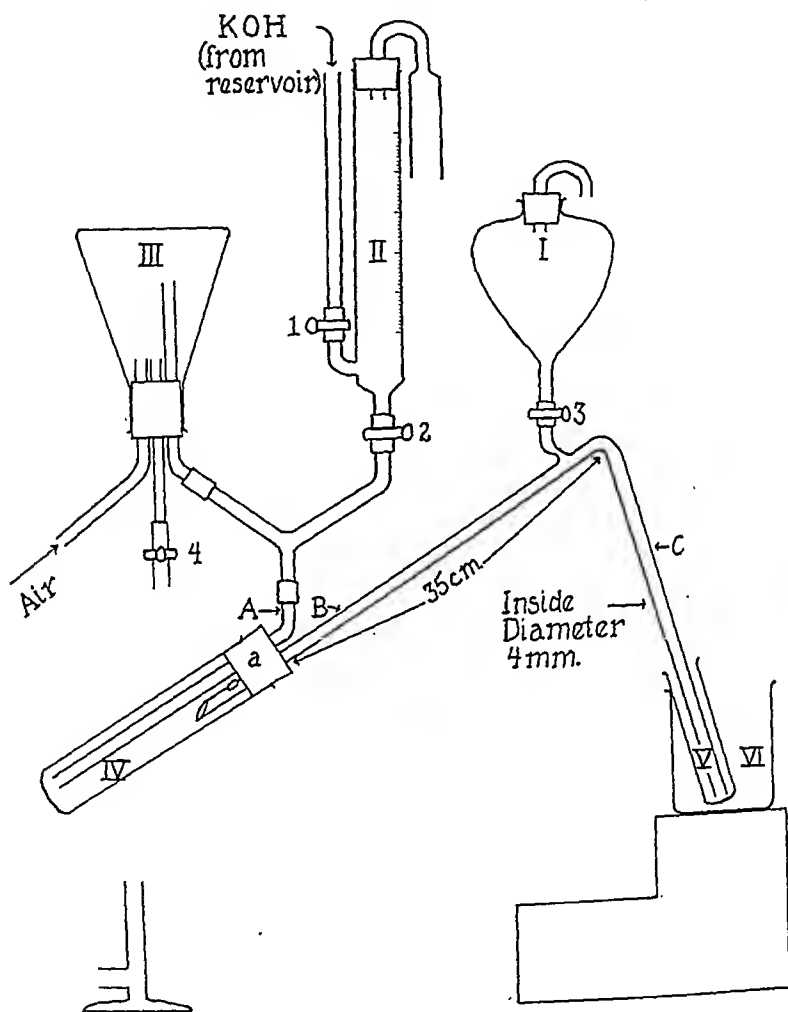


Fig. 1.

noise produced (about two minutes). At this point, remove the flame and open clamp *4*, stopping the flow of air. Lower the receiver until tube *C* is above the fluid level. If necessary, blow out tube *C* by momentarily placing a finger over the rubber tube below clamp *4*, but do not run a large amount of air through the apparatus. Wash back the ascending arm (tube *B*) of the distillation apparatus with water from *I* by opening clamp *3*. Cooling within the apparatus will suck the water back into *IV*. Now raise the receiver, start the air, and heat until steam again reaches the acid. Stop the distillation, and wash as before. Repeat this procedure once more. After the final washing of tube *B*, with the

receiver still in its lowered position, start the air and admit a small amount of water at clamp 3. This will be blown through C into the receiver. Again heat tube IV, and continue aeration and boiling for about two minutes. The total volume of the distillate and washings should be about 8 c.c.

DETERMINATION OF AMMONIA IN THE DISTILLATES

Total-, Albumin-, and Globulin-Plus Fibrinogen-Nitrogen. To the distillate add 3 drops of indicator and titrate with standard sodium hydroxide until no further change of color is produced by the addition of more alkali. We use a modified Rehberg⁸ burette of 1.0 c.c. capacity, calibrated to 0.01 c.c. The reading is estimated to 0.001 c.c.

Nonprotein Nitrogen and Fibrinogen. The distillate in the Peebles-Lewis colorimeter tube is made up to 8.0 c.c. with water. To this are added 2.0 c.c. of Nessler's solution, and comparison is made with standards prepared at the same time as the unknown solution. Two standards are prepared as follows:

	I	II
Ammonium sulfate	1.0 c.c.	2.0 c.c.
(0.04 mg. nitrogen per cubic centimeter)		
Water to make	16.0 c.c.	16.0 c.c.
Nessler's solution	4.0 c.c.	4.0 c.c.

In making the color comparison, the unknown is left at a volume of 10 c.c., and the standard is adjusted to match it. The volume required is recorded.

With extremely high values for nonprotein or fibrinogen nitrogen, it is necessary to use an aliquot of the distillate, therefore, it is advisable to add Nessler's solution to only one of each pair of duplicates until the approximate ammonia concentration has been determined.

DETERMINATION OF BLANKS

Two cubic centimeters of digestion mixture and 8 drops of 30 per cent hydrogen peroxide in a 25 by 200 mm. pyrex test tube are heated to dense fumes of sulfur trioxide. This is cooled well, and 10 c.c. of water are slowly added. Duplicate samples are distilled for titration and for nesslerization, using 8 c.c. of saturated potassium hydroxide. The distillate for nesslerization is made up to 8 c.c., and 2 c.c. of Nessler's solution are added. This is compared with a standard containing 1 c.c. of ammonium sulfate, 23 c.c. of water, and 6 c.c. of Nessler's solution. The value found is divided by 2 to obtain the correction blank.

We have found the ammonia blank for these reagents to equal the acid blank of 2 to 4 drops of indicator, and for convenience we have adjusted the amount of indicator between these limits to cancel the ammonia blank in the samples for titration.

CALCULATION OF RESULTS

Nonprotein Nitrogen:

$$\frac{\text{Reading of standard}}{20} \times \text{mg. nitrogen in standard} = \text{mg. nitrogen in distillate.}$$

$$\frac{(\text{Mg. nitrogen in distillate} - \text{blank}) \times 1.3 \times 100}{\text{Volume of plasma sample} \times 0.7} = \text{mg. nitrogen per 100 c.c.}$$

Fibrinogen. The calculation is similar to that for nonprotein nitrogen. The value for milligrams nitrogen per 100 c.c. is converted to milligrams protein by multiplying by the factor 6.25; this is divided by 1,000 in order to express the final result in terms of grams.

Total Protein:

$$\begin{aligned} &\text{c.c. N/10 sulfuric acid + indicator blank} - \text{c.c. N/10 sodium hydroxide} - \text{ammonia} \\ &\quad \text{blank} = \text{c.c. N/10 ammonia distilled.} \\ &\text{c.c. N/10 ammonia} \times 1.4 \times 100 \\ &\quad \text{Volume of plasma sample} = \text{mg. total nitrogen per 100 c.c.} \\ &\frac{\text{Mg. total nitrogen} - \text{nonprotein nitrogen}}{1,000} \times 6.25 = \text{Gm. protein per 100 c.c.} \end{aligned}$$

Albumin. The calculation is similar to that for total protein, except that the value for milligrams nitrogen must be multiplied by 2 since an aliquot equal to one-half of the albumin solution was used.

Globulin Plus Fibrinogen. The calculation is similar to that for total protein, except that no correction is made for nonprotein nitrogen.

REPRODUCIBILITY OF RESULTS AND A CONSIDERATION OF THE
"SALTING-OUT" PROCEDURE

The accuracy of the Kjeldahl procedure may be judged from the results obtained in a series of 24 analyses of the total nitrogen in 0.1 c.c. fractions of a single sample of plasma. The 24 determinations gave a mean value of 1.187 mg. nitrogen, with a standard deviation of 0.011 mg. nitrogen. The highest value obtained, 1.220 mg., is 0.033 mg., or three standard deviations above the mean, and the lowest value, 1.168 mg., is 0.019 mg., or about two standard deviations below the mean. The results obtained on a series of 100 consecutive complete determinations were also analyzed statistically.⁹ The standard deviation and standard error of the difference between duplicates for each fraction are given in Table I.

TABLE I
ACCURACY OF THE METHOD

NITROGEN FRACTION	STANDARD DEVIATION OF DIFFERENCE	STANDARD ERROR OF DIFFERENCE
	Gm. protein per 100 c.c.	Gm. protein per 100 c.c.
Total protein	0.072	0.101
Albumin	0.077	0.109
Globulin + fibrinogen	0.035	0.049
Fibrinogen	0.0087	0.0123
Nonprotein nitrogen	Mg. nitrogen per 100 c.c.	Mg. nitrogen per 100 c.c.
	1.123	1.742

In routine analyses a value obtained in a single determination should be within two standard deviations of the true value in 95 per cent of the cases. When duplicates are run, the results should differ by not more than two standard errors in 95 per cent of the cases. Thus, a single determination of the total protein by this method will give a value within ± 0.15 Gm. per 100 c.c. of the true value in 95 per cent of the analyses run. Similarly, it is unlikely that the value obtained for albumin is in error by more than ± 0.15 Gm.; for globulin plus fibrinogen, ± 0.07 Gm.; for fibrinogen, ± 0.017 Gm.; and for nonprotein nitrogen, ± 2.5 mg. per 100 c.c.

This concept of the accuracy of the method is based upon the assumption that an exact separation of the different protein fractions has been accomplished by the salting-out procedure. It is well known that a single "salting-out" will not quantitatively separate the two types of plasma protein, the albumin and globulin. In order to avoid this difficulty and still preserve the significance of analytical work, the terms "albumin" and "globulin" have come to mean those fractions of the plasma proteins which are separated by a single precipitation with sodium sulfate at a specific concentration and under controlled conditions. In the present work we have followed this accepted usage of the terms.

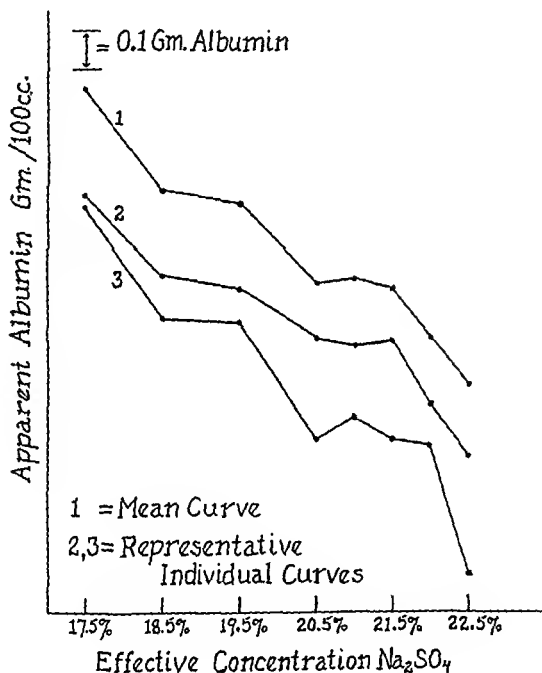


Chart 1.

Our choice of definitive conditions and concentrations of sodium sulfate is based largely upon the findings reported by Howe. This author¹⁰ has shown that the separation of fibrinogen with 10.6 per cent sodium sulfate gives values comparable to those obtained by other methods. In our work we have found that sodium sulfate in this concentration does not remove any of the protein from serum. It can, therefore, be concluded that no fraction other than fibrinogen will be separated from plasma unless it is adsorbed by the fibrinogen. In studying the protein precipitation with increasing concentrations of sodium sulfate,

Howe¹¹ found a critical zone between 20 and 22 per cent with plasma dilutions of 1:10. In our work the plasma dilution has been 1:20; however, this greater dilution has not produced a significant shift in the critical zone, as can be seen in representative curves given in Chart 1. In the curves of this chart an additional critical zone is shown at 18.5 to 19.5 per cent sodium sulfate, which was not found with some plasma samples. In all cases studied, covering a wide range of absolute values, the curves in the region of 21.5 per cent sodium sulfate were similar to those shown. Since this strength of sodium sulfate is usually employed for globulin precipitation, and does fall within the critical zone, we have continued to use a 21.5 per cent solution of the salt. Judging from the curves of Chart 1, it would seem that the use of 21.0 per cent sodium sulfate may be preferred, since this concentration falls more nearly at the center of the critical zone. It is probable that analytical results would not be significantly altered by this change. Although the separation of globulin and albumin is not complete, the values obtained on a given sample of plasma can be reproduced with considerable accuracy, and those on different samples of plasma should be comparable.

Chorine¹² has shown that the apparent plasma protein concentration may be markedly influenced by the nature and the amount of the anticoagulant used. According to this author's work, as well as that of others, heparin produces at most only a very slight change in plasma concentration. We believe, therefore, that our results give a true estimation of the amount of the circulating plasma proteins, unaltered by the anticoagulant which is added after the blood is drawn.

SUMMARY AND CONCLUSIONS

1. A method is presented for the separation and Kjeldahl analysis of the fibrinogen, albumin, and globulin of plasma.
2. A complete analysis of total nitrogen, nonprotein nitrogen, fibrinogen, albumin plus nonprotein nitrogen, and globulin plus fibrinogen may be done in duplicate on 0.8 c.c. of plasma.
3. A simple but accurate modification of existing micro-Kjeldahl procedures has been devised.

REFERENCES

1. Howe, P. E.: The Determination of Proteins in Blood, A Micro Method, *J. Biol. Chem.* 49: 109, 1921.
2. Robinson, H. W., Price, J. W., and Hogden, C. G.: The Estimation of Albumin and Globulin in Blood Serum. III. The Precipitation of Globulin at Twenty-Five Degrees by Sodium Sulfate, *J. Biol. Chem.* 126: 213, 1938.
3. Robinson, H. W., Price, J. W., and Hogden, C. G.: The Estimation of Albumin and Globulin in Blood Serum. I. A Study of the Errors Involved in the Filtration Procedure, *J. Biol. Chem.* 120: 481, 1937.
4. Harris, R. C.: The Adsorption of Protein by Filter Paper in the Estimation of Albumin in Blood Serum, *J. Biol. Chem.* 127: 751, 1939.
5. Howe, P. E.: The Relative Precipitating Capacity of Certain Salts When Applied to Blood Serum or Plasma and the Influence of the Cation in the Precipitation of Proteins, *J. Biol. Chem.* 57: 241, 1923.
6. Lewis, R. C.: Two Improved Designs of the Peebles-Lewis Clinical Colorimeter, *J. LAB. & CLIN. MED.* 16: 914, 1931.
7. Goebel, W. F.: Quoted in Peters, J. P., and Van Slyke, D. D.: Quantitative Clinical Chemistry, Baltimore, 1932, Vol. II, page 531, Williams & Wilkins Co.

8. Rehberg, P. B.: A Method of Microtitration, *Biochem. J.* 19: 270, 1925.
9. Mainland, D.: *The Treatment of Clinical and Laboratory Data*, London, 1938, Oliver & Boyd, page 321.
10. Howe, P. E.: The Determination of Fibrinogen by Precipitation With Sodium Sulfate Compared With the Precipitation of Fibrin by the Addition of Calcium Chloride, *J. Biol. Chem.* 57: 235, 1923.
11. Howe, P. E.: The Use of Sodium Sulfate as the Globulin Precipitant in the Determination of Proteins in Blood, *J. Biol. Chem.* 49: 93, 1921.
12. Chorine, V.: Influence des Anticoagulants sur le Dosage des Éléments du Sang, *Ann. Inst. Pasteur* 63: 213, 367, 1939.

AN IMPROVED RAT CAGE FOR METABOLISM STUDIES*

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DURING the last few years the well-known wire drum and glass funnel metabolism cages have been in use in this department, but just as in other laboratories, we found that this cage had many disadvantages (contamination of feces and urine with food, washing of feces by urine, evaporation of urine, etc.). In the course of time many improvements have been made by members of this laboratory, using similar cages for metabolic studies, and gradually an entirely new type of cage was developed which eliminates most of the disadvantages mentioned. Since this metabolism cage proved very satisfactory, we wish to describe it here briefly in the hope that it will prove useful to other investigators as well.

Fig. 1 shows the complete unit of six cages with all parts assembled, as it appears when in use.

Fig. 2 shows the individual parts:

Cage. 7 by 8 by 5 inches of galvanized iron with wired top edge, having a hinged wire mesh door or lid (4 to 1 inches) fastened by means of a bronze door clip. The base of the cage is of wide wire mesh as indicated, to allow feces to drop through. Two outlets $1\frac{3}{8}$ inches in the front end of the cage enable the rat to obtain food and water. These holes must be placed high enough in the wall of the cage so that it becomes necessary for the rat to place its front paws on the edge of the hole when it takes food. This arrangement prevents the animal from lifting food into the cage where it would become mixed with excrements. Therefore, the height of the holes must be chosen according to the size of the rat. Above, center, is a small hole into which fits a rivet on the diet box which holds the box securely fastened to the cage front. An angle rest (as shown), front and back, supports the cage on the rack.

Diet Box. This box $5\frac{1}{2}$ by $2\frac{1}{8}$ by 4 inches of galvanized iron is divided into two compartments. One forms a food bin of wire mesh for gross food; the other, with a dividing shelf with hole in center, allows tip of water bottle to pass through; this shelf acts as a support for the bottle and prevents the eating of

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the rubber stopper by the rat. Below these two compartments is sufficient space to insert a two-compartment drawer which is used to hold ground food on one side and to catch drips from the water bottle on the other. If extreme accuracy in determining of food intake is required, only ground food should be admin-

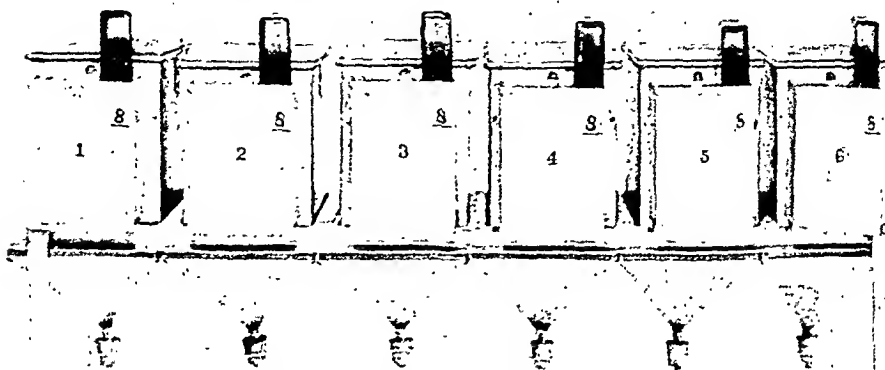


Fig. 1.

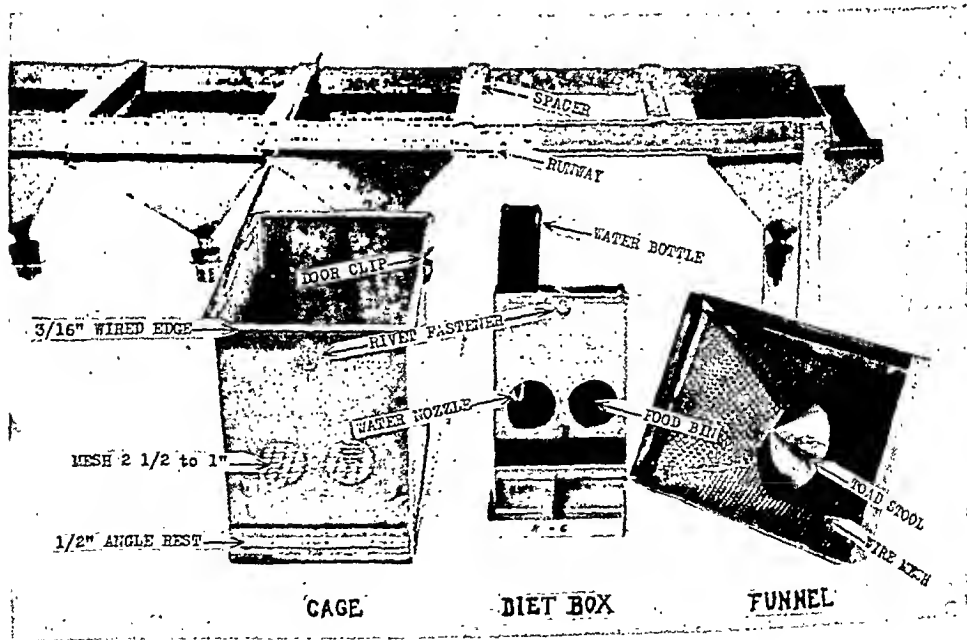


Fig. 2.

istered, since this can be measured exactly before and after the experiment by weighing the contents of the drawers. When the animal is being fasted, a small tray replaces the food drawer, fills in the space, and prevents the rat from crawling through the outlets into the diet box and the consequent loss of urine.

Rack. Legs are of $\frac{3}{4}$ by $\frac{3}{16}$ inch flat bar welded to 1 by $\frac{1}{2}$ inch angle iron; runway for funnels of 1 by $\frac{1}{2}$ by $\frac{1}{8}$ inch angle iron extending from end to end of the rack. Flat bar spacers $\frac{3}{4}$ by $\frac{1}{8}$ inch are welded to side bars front and back, spaced along the length of the rack at a distance of the width of each cage to prevent cages from sliding out of position over the funnels.

Funnels. Constructed of monel metal throughout are pyramidal in shape. Inserted in each funnel is a monel wire mesh rack similar in shape but somewhat less deep than the funnel and having a monel "toad stool" in the center. The toad stool is so constructed that the feces dropping from above roll off and collect in the rack at the base. The urine runs off the stool and drips directly through the mesh into the deeper funnel below without washing the feces. The funnel drains into an Erlenmeyer flask fastened by means of an airtight rubber stopper placed over the end of the funnel drip tube. This arrangement keeps evaporation of urine at a minimum.

As will be seen a supporting rivet is placed on both sides of the diet box; the use of one has already been described. The second is used when both fasting and thirst are desired. In this case it is possible to turn the diet box around, thus presenting a solid front toward the rat and covering the outlets of the cage.

The main advantages of this cage are:

- (1) No contamination of feces and urine by food.
- (2) No washing of feces by urine.
- (3) Achievement of extreme accuracy in measurement of both food and water intake.
- (4) Minimum urine evaporation.
- (5) The cages are relatively inexpensive because they are unbreakable and rust proof.

MEDICAL ILLUSTRATION

THE PHOTOGRAPHIC ASPECT OF LIGHT REFLECTION FROM HUMAN SKIN*

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CONSIDERABLE study has been given to the transmission and penetration of light *through* human skin, but it would appear from the literature that little attention has been given to the photographically important question of *reflection* of light from human skin. Experience reveals wide variations in the photographic appearance of skin taken under different conditions. (1) Such variations are attributed to the color sensitivity of the emulsion used, the color temperature of the light source, and/or the use of color filters. These explanations are inadequate since they place the subject on a par with the photography of colored objects and their translation into monochrome; they do not account satisfactorily for the need to use make-up to secure good photographs in color of the human face, and still less do they explain the cause of the variations in texture and definition shown by photographs of human skin taken by reflected light of different color temperatures. (2) More particularly, they afford no understanding of why skin photographs are waxlike under red light and show crisp definition under blue light.

The thesis of this paper is that human skin is a living tissue with definite characteristics with regard to the response of different wave lengths to light, and that the true explanation of the differences shown in photographs is to be found by ascertaining the *level* from which reflection of light is taking place. The basic point of this thesis is that different wave lengths of light are reflected from different levels (depths) of human skin, and this introduces four additional factors: penetration, absorption, reflection, and scatter, consideration of which is necessary for a complete explanation of the results obtained.

Skin varies considerably in thickness in different parts of the body, e.g., cross sections from the back show greater thickness of the stratum mucosum than similar cross sections from the back of the hand (Fig. 1, layer C). Similarly, skins from the palm of the hand or sole of the foot vary in thickness, and there is thus no one area standard for the whole body. For the purpose of experiment, skin from the back of the human hand was used throughout.

Fig. 1 is a photomicrograph of a transverse section of skin from the back of a hand of a Chinese and shows its structure and various layers. On it are shown the approximate levels at which it would appear by experimental photo-

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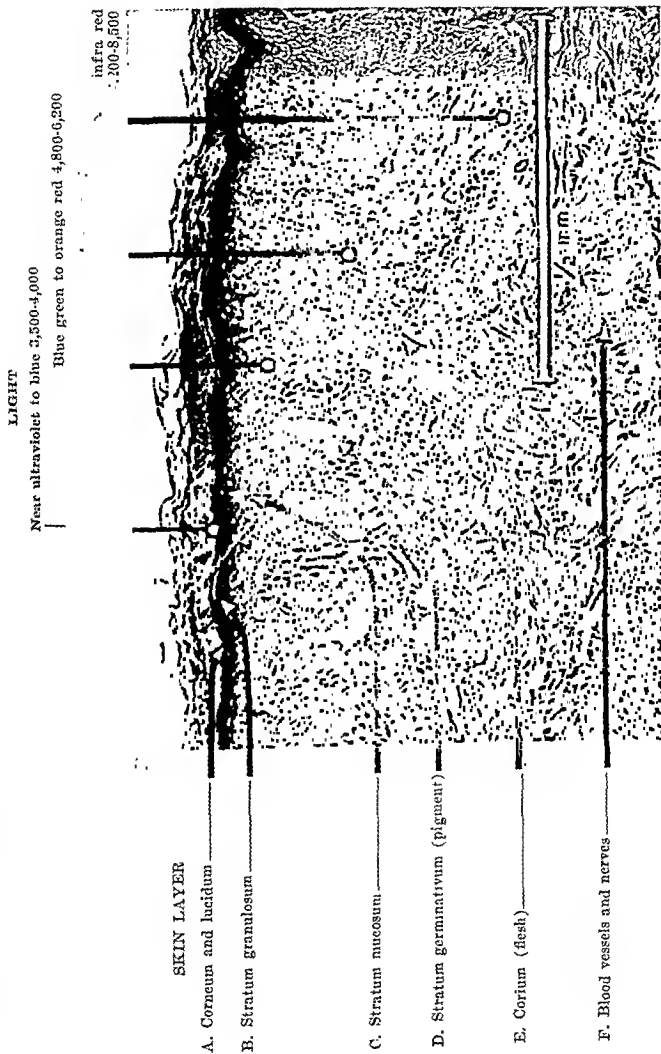


Fig. 1.—Photomicrograph of a transverse section of skin from the back of the hand of a Chinese, showing its structure and various layers. On it are seen the approximate levels at which it would appear by experimental photographs that the light used was reflected.

graphs that the light used was reflected. Such levels must necessarily be approximate only, because, as previously stated, human skin in any event varies in thickness.

Experiments in photographing through skin and flesh indicate² that a powerful beam of light has considerable penetrating power and that, as is logical, the stronger and more concentrated the light, the greater the penetration and transmission. Such experiments further reveal that the transmission is mostly toward the red end of the spectrum and progressively decreases as it approaches violet and ultraviolet.³⁻⁸ This confirms results obtained photographically by reflected light.

TABLE I

FIGURE NO.	HAND	PLATE USED	ILLUMINATION USED	FILTER USED
2	European	Special rapid	Quartz mercury vapor	Tri-blue
3	European	Special rapid	Half-watt	Tri-blue
4	European	Panchromatic	Half-watt	None
5	European	Panchromatic	Half-watt	Beta
6	European	Panchromatic	Half-watt	Tri-red
7	European	Infra-red	Half-watt	Infra-red
14	Indian	Special rapid	Quartz mercury vapor	Tri-blue
15	Indian	Infra-red	Half-watt	Infra-red
8	European and Chinese	Infra-red	Half-watt	Infra-red
9	European and Chinese	Panchromatic	Half-watt	Tri-red
10	European and Chinese	Panchromatic	Half-watt	None
11	European and Chinese	Special rapid	Half-watt	Tri-blue
12	European and Chinese	Special rapid	Quartz mercury vapor	Tri-blue
16	Case of pellagra, Chinese	Special rapid	Daylight	Tri-blue
17	Case of pellagra, Chinese	Panchromatic	Daylight	None
18	Case of pellagra, Chinese	Infra-red	Daylight	Infra-red

To demonstrate the correctness of our thesis a series of photographic experiments was carried out, using the following Ilford plates and filters, with a quartz mercury vapor lamp, half-watt lamps, and daylight. The plates used were:

Ilford "special rapid," an ordinary or nonecolor-sensitive plate of medium speed, sensitive from about 3,600 to 4,950 A.U., which is near ultraviolet to greenish blue. With this plate an Ilford tri-color blue filter cutting at 5,150 A.U. was used; thus, near ultraviolet and all the blue were used for taking the photograph.

Ilford "special rapid panchromatic," an orthopanchromatic type with the same development constant as the "special rapid," but sensitive from about 3,600 to 7,200 A.U. With this a beta filter was used once as a correction filter, because with half-watt light this gives practically full correction. This filter is a pale greenish yellow with a factor to H.W. of $\times 1\frac{1}{2}$ only.

Ilford "infra-red," an infra-red sensitive plate which, when used with its appropriate filter, records the action of rays between 7,000 and 9,000 A.U., with maximum sensitivity at about 8,100 A.U. The filter permits scarcely any

visible light to pass, and it is necessary to focus by a deep red filter and compensate by a slight increase in bellows extension when using it. The exact amount is $1/200$ of the focal distance of the lens.

All plates were backed and thus proof against halation. A Goerz Dagor F:6.8 - $8\frac{1}{4}$ inch lens was used, stopped down to F:22. Figs. 2 to 12 were taken with a vertical camera in a darkened room without any other light than that indicated; Figs. 16 to 18 were taken in a hospital ward by daylight, the patient being in bed and too ill to be moved. Development of the special rapid and special rapid panchromatic plates was routine, a developer of the D.76 type being used, with time and temperature for contrast of about 0.9 gamma. Exposures were based on meter readings, and prints were made on Kodak "Nikko" paper of medium contrast; this corresponds to grade 2; P.M.C. paper. No attempt was made to depart from the usual procedure and no contrasts were forced up or down by interference with normal development or printing.

Table I lists the experiments, and the illustrations show the photographic differences in skin-rendering.

RESULTS

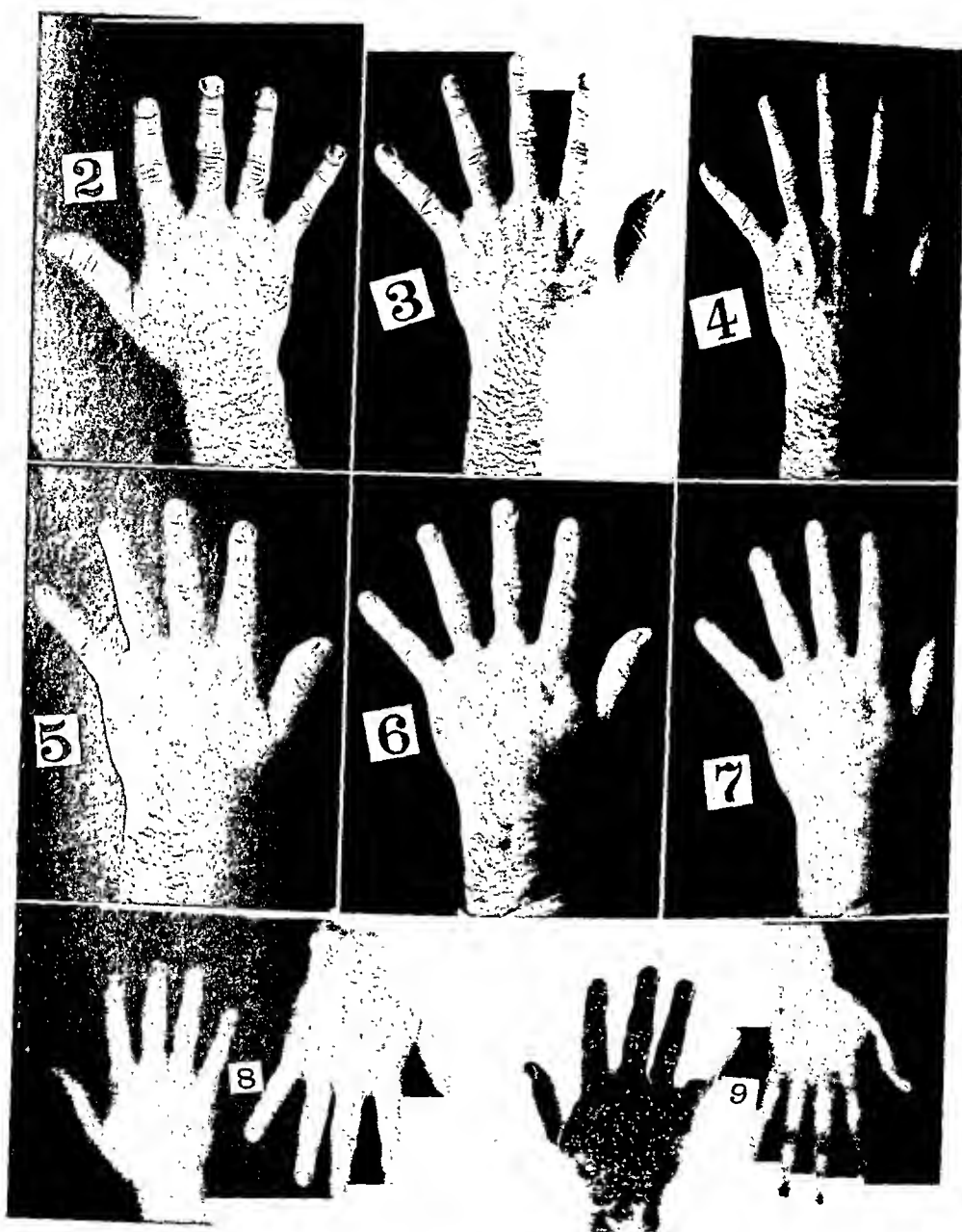
Figs. 2 to 18 show definite changes in the photographic rendering of skin texture. The longer the wave lengths employed, the greater the loss of fine detail in the skin. The results are set forth here as a record because it is impossible for a half-tone reproduction to show the gradual changes as clearly as can be seen in the originals.

FIG.

APPEARANCE

- 2 Minutest lines and details of skin texture are clearly recorded; more is recorded than can be seen in daylight by the eye aided by a low-power magnifying glass.
- 3 This shows excellent rendering of texture which is slightly inferior to Fig. 1.
- 4 The general appearance is excellent, but the texture is not as crisp as in Fig 1.
- 5 The skin is commencing to show less texture.
- 6 The typical waxy appearance of the skin is caused by excess red light; the texture is poor.
- 7 Texture has completely disappeared; subcutaneous veins are visible.
- 14 This excellent skin texture shows the finest details.
- 15 There is complete absence of surface texture of the skin; underlying veins show through.
- 8 No texture can be seen but the veins are clearly visible.
- 9 Shows waxy appearance, with little texture and detail.
- 10 The general appearance is excellent, but crispness of lines is lacking.
- 11 Crispness of lines has reappeared, especially in the skin of the Chinese.
- 12 Minutest lines and details of skin texture are shown. Note the higher absorption of short waves by the hand of the Asiatic compared with that of the European.

The end differences shown are great. To make direct comparison between photographs taken by blue and by red light more simple, as occurs in tri-color separation negatives of human skin, the portion of skin around the knuckles



- Fig. 2.—Ultraviolet on special rapid plate.
 Fig. 3.—Tri-color blue on special rapid plate.
 Fig. 4.—Panchromatic, half-watt, no filter.
 Fig. 5.—Panchromatic, half-watt, beta filter.
 Fig. 6.—Panchromatic, tri-color red filter.
 Fig. 7.—Infra-red hand of European
 Fig. 8.—Infra-red, European and Asiatic.
 Fig. 9.—Tri-red on panchromatic, European and Asiatic.

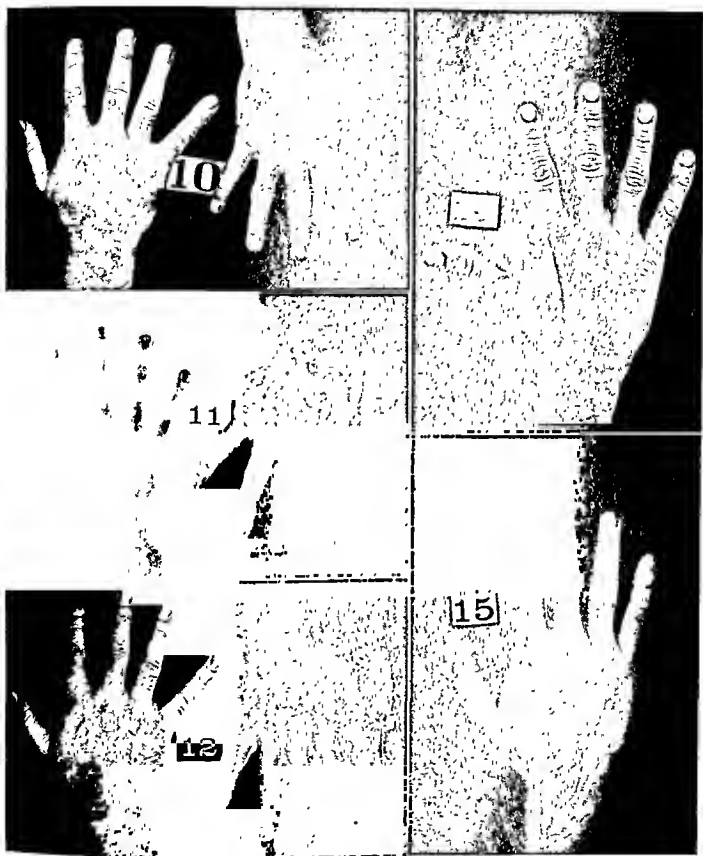


Fig. 10.—Half-watt with panchromatic, European and Asiatic.

Fig. 11.—Tri-color blue, European and Asiatic.

Fig. 12.—Ultraviolet, European and Asiatic.

Fig. 14.—Ultraviolet, Indian hand.

Fig. 15.—Infra-red, Indian hand.



See legends on bottom of opposite page.

of the middle and third fingers in Figs. 3 and 6 have been enlarged and are shown in Figs. 19 and 20. These are not the extremes, which would be Figs. 2 and 7, 14 and 15, and 9 and 12. Figs. 3 and 19 show the approximate effect secured in normal sunlight by using an ortho film, whereas Figs. 6 and 20 are those secured on a hypersensitive or type 3 panchromatic film by nitro-phot lights, or lights abundant in red rays.

These two comparisons are, therefore, the most important from a practical photographic aspect, since the conditions are commonly met with in ordinary practice.

DISCUSSION

The photographs reveal that as the wave length of light used for illumination is increased, the surface texture that can be photographed is progressively diminished and finally entirely lost. This can only mean that reflection of the rays that are actually making the photographic negative is taking place from different levels of the skin. Therefore, the photographic rendering of human skin is not solely a matter of color correction and color sensitivity, but is closely connected with the inherent response of skin as a living tissue to light thrown upon it.

Figs. 16, 17, and 18 are comparison photographs of the skin condition on the hands of a patient with pellagra. The panchromatic version (Fig. 17) is a good representation of the visual appearance. The blue plus near ultraviolet version (Fig. 16) accentuates surface lesions and reveals more than the eye sees; the infra-red version (Fig. 18) shows that the true extent of serious damage is limited to the fingers. It is obvious that such photographs are of great diagnostic value.

Table II sets forth an interpretation of the results of the experiments.

TABLE II

LIGHT	ABSORPTION	REFLECTION LEVEL	REFLECTION INTENSITY	SCATTER
Near ultraviolet to blue	4	A to B	1	1
Blue green to orange	3	B to C	2	2
Red	2	D	3	3
Near infra-red	1	E and F	4	4

The numbers 1 to 4 are arbitrary indicators for the amount absorbed, reflected, and scattered, as follows: (1) minute; (2) little; (3) moderate; (4) considerable. For practical purposes it is sufficient to divide the light into these four divisions, and in any case the varying thickness of skin makes exact measurements valueless.

Legends for Figs. 16-20.

Fig. 16.—A case of pellagra in a Chinese taken on a special rapid plate with daylight illumination.

Fig. 17.—A case of pellagra in a Chinese taken on a panchromatic plate with daylight illumination.

Fig. 18.—A case of pellagra in a Chinese taken on an infra-red plate with daylight illumination.

Fig. 19.—This illustration is an enlargement of the center section of Fig. 3. It was taken on a special rapid plate through a tri-blue filter with the half-watt light as illumination.

Fig. 20.—An enlargement of the center section of Fig. 6. This was taken on a panchromatic plate through a tri-red filter with the half-watt light as illumination.

It was mentioned earlier that the stronger and more concentrated a beam of light, the greater its transmission through skin and flesh. This introduces yet another factor, the strength or volume of light employed on a given area. The latter has a distinct effect on photographic rendering. This would appear to furnish one of the reasons why overlighting is disastrous to good photographs of skin texture, since evidently it results in abnormal penetration of light into the skin, with a consequent accentuated reflection from the lower layers causing excessive scatter.

Further, these experiments show clearly that the modern hypersensitive panchromatic film (type C) used in conjunction with half-watt light is not the ideal combination for work on skin. A study of the old photographs published in textbooks reveals that in general they are superior as records of skin to some more recent examples. In this sense there has been retrogression in the technical photography of skin texture.

In photographing human skin for medical records it is of paramount importance that the visual appearance of the texture and skin lesions present be clearly recorded. This is particularly necessary when a specific pathologic condition is to be illustrated, for unless the diseased condition is plainly shown the photograph is worthless.

Both normal and diseased skins have texture, but the recording of this texture is not achieved photographically unless basic requirements to secure it are known and fulfilled. Medical photography is decidedly different from portraiture; the latter aims at the suppression of defects which it is the precise function of the biologic photograph to record. The approach to the two types of subject is, therefore, entirely different.

The surface of human skin is covered with a multiplicity of fine criss-crossed lines, thus giving it the marked texture characteristic and typical of its appearance. Skin is a good reflector of normal daylight; it reflects more of the longer waves and absorbs more of the shorter, and strongly polarizes light reflected between the angles of 32 and 37 degrees. The elimination of reflections by polarizing screens destroys its living appearance and affects the recording of texture; this is evidence that it is solely the reflected light which governs the photographic appearance of skin.

The two basic factors are thus inequalities of surface and variations in reflection. Illumination must take both into consideration and so light the subject that the minute hills and dales of the skin surface are not flattened by being flooded with light. The quality of light must be such that reflection is taking place from some known portion of the skin, that is, at some known position with regard to its surface. The first requirement is standard for all photography; illumination must be so arranged that the subject is shown properly. The second is generally ignored, but is of fully as great importance. It is generally assumed that the problems are much the same as with portraiture and that, provided the lighting is directionally correct and a suitable emulsion is used, results will be as good as can be secured. This is not so.

Light thrown on human skin penetrates it, and is absorbed and reflected by it, the degree of penetration, absorption, and reflection depending upon its component wave lengths. The photographic definition secured in a negative

is considerably affected by the amount of penetration taking place and the consequent depth of reflection from the inner surfaces of the skin. This has little connection with color-rendering into monochrome as such, for the problems are specific to human skin.

Penetration plus reflection obviously causes scatter, depending upon the depth of the intervening layers of skin which have been traversed by light. The deeper this penetration, the more scattering will be the reflected rays, since such rays are scattered not only on entering but also on being reflected back to the surface; hence the difference in appearance of the same skin photographed by light of different spectral quality.⁹ It is clear that an understanding of the cause of this difference enables the securing of exactly the type of photograph required.

It can be accepted as a fact that penetration through skin of the light of the complete visible spectrum, which for the purposes of this paper is taken to include the near ultraviolet and near infra-red rays, is greater as the wave length of the light increases.⁸ Much work has been done along these lines, with interesting results confirmed by practical photographic experience.⁴ Normally, photography is entirely dependent upon reflected light; therefore, the reflection levels rather than absorption and transmission are photographically of greater importance.

Light has a threefold action on impact with skin: absorption, penetration, and reflection. Photographically, light absorbed by a subject is lost and of no value. Since the longer wave lengths penetrate more deeply, those that are not absorbed must necessarily be reflected back to the surface from the maximum depth of penetration, and this is the light used to make the photograph and govern the sharpness of the image secured. That is, other things being equal, the photographic rendering of skin is fixed definitely by the quality of light used in the making of the photograph, and it is impossible to change this fundamental record by any subsequent chemical tinkering with the negative or print. It should now be clear that it is not a matter of color-rendering as such, because texture and definition have no direct connection with the interpretation of color into monochrome.

Although absorption is outside our subject, it is photographically important because it not only affects exposure time but also governs the color-rendering. When there is much absorption, the subject photographs dark, and vice versa. A good example of this is green vegetation photographed by blue light (or on a noncolor-sensitive emulsion) as compared to the same subject photographed by infra-red. Green leaves absorb ultraviolet and deep blue but reject deep red and infra-red; therefore, the first photograph will show the subject dark and the second will show it light in tone.^{10, 11} An even more striking example is a comparison between the brown fur of a dog and the brown feathers of birds; visually there is little difference in color, but photographically there is a wide difference in exposure time required owing to the absorption of ultraviolet by brown fur and the strong reflection of ultraviolet by feathers. Measurements with photoelectric meters reveal this difference and the camera confirms it. Nature has given the animals protection against receiving insufficient or too large doses of ultraviolet. The bird is exposed to too much while flying in the

sky, whereas the animal needs all the chemical rays it can get to maintain its supply of vitamin D. There is unquestionably a similar protective system at work in the skin of man, which accounts for the selective absorption of light and its variation in the different races of mankind according to the climatic conditions under which he lives.

There are three methods of controlling the quality of light used in the making of a photograph:

1. The interposition of a light filter at some point between the light source and the sensitive film.
2. The use of light sources rich or deficient in certain wave lengths.
3. Selection of the rays by the choice of film.

The final negative quality and photographic rendering of skin will be strongly affected by these fundamental factors. It is notorious that the use of modern extreme, red-sensitive emulsions with artificial light abnormally rich in the longer wave lengths gives a waxy, textureless appearance to the skin, and our contention is that the reason for this is reflection from beneath the skin surface.

It is an interesting speculation that there may be an interference effect at the skin surface which may produce *stationary waves* somewhat along the lines of the Lippmann process of color photography.¹²

SUMMARY

The photographic appearance of human skin is modified by the reflection depths of the component wave lengths of the light used for illustration, and as such it is not a matter of color correction, as ordinarily understood, but the level of penetration and consequent reflection and scatter of the light used. Evidence is offered in the form of a series of photographs taken by various illuminants.

I wish to thank Professor Bernard E. Read, acting director of the Henry Lester Institute of Medical Research, for his help in the writing of this paper, and Dr. L. S. Kau, Tissue Pathologist of the Institute, for supplying the sections of skins required.

REFERENCES

1. Dent, R. V.: The Photographic Illustration of Medical Subjects, *Photographic J.*, April, 1938, p. 201.
2. Ibid.: p. 205, Figs. 8 and 9.
3. Laurens, Henry: The Physiologic Effects of Ultraviolet Radiation, *J. A. M. A.* 111: 2386, 1938.
4. Bachem, A., and Reed, C. I.: The Penetration of Light Through Human Skin, *Am. J. Physiol.* 97: 86, 1931.
5. Rawling: Infra-Red Photography, p. 49.
6. Russell and Russell: Ultraviolet Radiation and Actinotherapy, pp. 272-273, 280.
7. Cartwright, Daniel, and Petruskas: Penetration of Light Through Cheek, *Science, Supplement*, 93: No. 2406, February 7, 1941.
8. Laurens, Henry: Physiological Effects of Radiant Energy, p. 101.
9. Helwich: Practical Infra-Red Photography, p. 85.
10. Atkins, W. R. G.: The Transmission of Light and Total Radiation by Leaves, *Proc. Roy. Soc.* p. 26, March, 1937.
11. Bloch, Olaf: Recent Developments in Infra-Red Photography, *J. Roy. Soc. Arts* 81: 5, 1933.
12. Hardy and Perrin: Principles of Optics, p. 574.

DEPARTMENT OF ABSTRACTS AND REVIEWS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

SULFAPYRIDINE, Level of in Blood of Children Following Dosage by Weight, Cullen, G. E., and Wilson, A. T. *Am. J. Dis. Child.* 60: 891, 1940.

A simple and convenient standard system of dosage of sulfapyridine on the basis of body weight is recommended: 1 grain per pound of body weight per twenty-four hours, with an initial dose of half the calculated twenty-four-hour dose and with the total daily dose not to exceed 80 grains.

Groups of infants and children of different ages have, on the average, approximately the same levels of free sulfapyridine in the blood when they are given the same dosage according to body weight.

Individual patients receiving sulfapyridine in a dosage based on body weight show only moderate fluctuations in the levels of the drug in their blood from day to day or throughout a single day.

The averages of several individual values of patients receiving sulfapyridine in a dosage by body weight vary widely from patient to patient.

In view of the several types of rather marked and unpredictable variations, it must be concluded that it is desirable to make frequent determinations of the actual level present in any given patient and subsequently to adjust the dosage according to the level desired.

URINARY TRACT, Chemotherapy in Nonspecific Infections of, Alyea, E. P., and Roberts, L. C. *J. A. M. A.* 115: 1345, 1940.

The sulfonamide drugs are excreted by the kidneys in a manner exactly similar to phenolsulfonphthalein.

In vitro and in vivo studies show the specificity that the sulfonamide drugs have for different bacteria and different strains of the same bacterium.

Experimental studies in vitro are not necessarily entirely comparable in vivo.

The action of sulfonamide drugs in infections of the urinary tract depends more on the tissue reaction than on direct bactericidal action in the urine.

Mandelic acid is an excellent drug for infections with colon bacilli and *Streptococcus faecalis*.

A comparison of the colon bacillus infections treated with sulfanilamide and sulfapyridine shows practically the same, or 81 per cent, cured.

A comparison of the same drugs in staphylococcal infections shows that with sulfapyridine 75 per cent were cured and with sulfanilamide 62.5 per cent.

Response to the sulfonamide drugs is rapid, usually within two or three days.

Infections complicated by other pathologic changes do not respond as favorably as the simple infections.

A comparison of sulfanilamide and mandelic acid therapy in various types of cases shows that sulfanilamide is usually preferable.

Evidence shows that the high drug concentration in the urine usually thought desirable is not necessary for cures.

A dosage of 1.7 Gm. of sulfanilamide a day with forced fluids produced as good results as 3 Gm. a day with restricted fluids. The same is true with sulfapyridine.

Many patients cannot take the large doses with restricted fluids, but the recommended small dosage is easily tolerated by all.

RENAL FUNCTION: Relative Significance of Concentration of Inorganic Sulfate in the Serum and of Its Renal Clearance, Goudsmit, A., Jr., and Keith, N. M. Arch. Int. Med. 66: 816, 1940.

Comparative studies of blood urea, urea clearance, serum sulfate, and sulfate clearance were performed on 50 patients. The majority were suffering from diffuso arteriolar disease with hypertension of various degrees of severity; most of the remainder, from chronic glomerulonephritis and other diseases of the urinary tract. The selection of the patients was conducted in such a manner that half of the group would have normal and the other half increased concentrations of sulfate in the serum.

The increased concentration of serum sulfate was found to occur in diffuse arteriolar disease with hypertension groups 3 and 4 in 4 of every 5 cases, the urea clearance, the value for blood urea, and the sulfate clearance indicating renal damage with decreasing frequency. The correlation of renal damage sufficient to bring about actual retention of an end product of normal protein metabolism and hypersensitive vascular disease severe enough to be associated with retinitis is significant. It is assumed that arteriolar disease may well be responsible for both the retinal and the renal changes.

Generally speaking, in the more severe degrees of hypertension, as contrasted with the milder forms, there is a far greater probability of retention of either urea or sulfate in the blood than of decreases in the renal clearance of these substances. In this respect the patterns of excretion of urea and of sulfate are essentially similar. However, whereas the association of retention of urea in the blood and a normal value for urea clearance was observed in only one instance, 12 instances of increased serum sulfate in the presence of a normal value for sulfate clearance were observed.

The increase of the concentration of serum sulfate itself is held responsible for this behavior, and an analogy is seen with observations on experimental animals as well as on human volunteers, in whom the intravenous injection of sodium sulfate leads to considerable increases in the value for sulfate clearance without significantly changing renal function otherwise. In accordance with the analogy drawn from these experimental observations, the ratio of sulfate clearance to urea clearance is significantly increased in the cases of those patients who have increased values for (endogenous) sulfate in the serum as compared to those with normal values for serum sulfate.

Thus, although conclusions of prognostic significance in renal disease should not be based on the value for serum sulfate any more than on any other single laboratory index, determination of this index is a valuable adjunct in appraising renal function, and the pattern of excretion of sulfate is of considerable interest to the student of renal and vascular disease.

BLOOD SUGAR, Normal Values in Children, Rudesill, C. L., and Henderson, R. A. Am. J. Dis. Child. 61: 108, 1941.

A total of 288 blood sugar determinations were made by the Folin-Wu method on 144 nondiabetic children from 2 to 15 years of age, inclusive. Values for both fasting and 11 A.M. blood sugar were determined on the same day and the reports were correlated.

There is no practical difference between fasting and 11 A.M. blood sugar values, since both fall within normal limits.

If the Folin-Wu method is used, normal fasting blood sugar values in nondiabetic children from 2 to 15 years of age range from 70 to 105 mg. per hundred cubic centimeters.

A review of the literature indicates that the range of normal blood sugar values in infants is probably from 60 to 100 mg. per hundred cubic centimeters in most cases.

STAPHYLOCOCCI, Technic of, The Coagulase Test for, Fisk, A. Brit. J. Exper. Path. 21: 311, 1940.

Within wide limits the amount of sodium citrate does not materially affect the coagulase test.

Citrated plasma diluted 10 to 20 times gives satisfactory results.

Human plasma from different individuals does not show wide variations in clotting time.

The citrated plasma remains suitable for the test for several weeks if kept undiluted under sterile conditions.

Staphylococci growing in a variety of media produce coagulase. Of a number of liquid media, broth gave a culture which produced coagulation most quickly. Agar cultures may also be employed, in which case it is best to emulsify the organism in broth to form an even suspension of an opacity approximately equal to that of a twenty-four-hour broth culture.

There is an optimum ratio between amount of organism and amount of plasma.

The reaction occurs most quickly at 37° C.

The method suggested for the performance of the coagulase test is as follows:

1. Dilute citrated human plasma tenfold with normal saline.
2. Place 0.5 ml. of this diluted plasma in two small test tubes.
3. To one of these add 5 drops from a capillary pipette (approximately 0.125 ml.) of an overnight broth culture of staphylococcus or a suspension of an agar culture made in broth to an opacity approximately equal to an overnight broth culture. The second tube serves as a control and must show no clotting. Incubate the tubes at 37° C.
4. Examine the tubes after half an hour and at intervals for six hours. (Clotting of the plasma usually occurs within one hour.)

STAPHYLOCOCCI, Use of Slide Agglutination to Determine Pathogenicity of Staphylococci, Christie, R. Australian J. Exper. Biol. & M. Sc. 18: 397, 1940.

A total of 335 strains of staphylococci of human origin and 57 strains of animal origin have been examined.

Of 269 coagulase-positive strains, 257 were agglutinated by a mixture of three antisera prepared from coagulase-positive strains; nine strains were auto-agglutinable and could not be tested. One human and two animal strains were not agglutinated; the human strain was serologically related to the coagulase-positive strains in spite of its inagglutinability with the serum mixture. Of 123 coagulase-negative strains, 120 were not agglutinated, two were auto-agglutinable, and one was agglutinated slightly.

An attempt to produce agglutinating sera with coagulase-negative strains which would agglutinate coagulase-negative and not coagulase-positive strains was unsuccessful.

HYPOPROTHROMBINEMIA, Intravenous Use of 2-Methyl-1, 4-Naphthoquinone in, Norcross, J. W., and McFarland, M. D. J. A. M. A. 115: 2156, 1940.

2-Methyl-1, 4-naphthoquinone has been administered intravenously to 22 patients. It has proved to be efficient in raising the prothrombin level. Bile salts were not necessary for this response.

With the exception of local distress for short duration at the time of injection, no toxic manifestations have been observed.

The minimal effective intravenous dose of 2-methyl-1, 4-naphthoquinone in the adult was found to be 2 mg.

In cases uncomplicated by liver impairment a 2 mg. dose given intravenously raised the prothrombin level from 50 per cent to 80 per cent in an average of seven and one-half hours and to 100 per cent in an average of twenty hours.

A 2 mg. intravenous dose kept the prothrombin level above 90 per cent of normal for four or more days unless liver damage was present.

Severe liver damage permitted only a slight response to intravenous 2-methyl-1, 4-naphthoquinone. If liver impairment was less marked, the effect was manifested in a slower onset of response, a lessened height of response, and a shorter duration of response.

Bleeding was rapidly and effectively controlled unless severe damage was present.

The intravenous route of administration has great value in patients for whom oral administration is difficult or impossible, as well as in those in whom rapid cessation of bleeding is imperative.

PNEUMONIA in Children, Treatment of, With a Single Dose of Sulfapyridine, Platt, L.
Am. J. Dis. Child. 60: 1019, 1940.

In the children included in this study the single-dose method gave satisfactory levels in the blood in most instances, and the clinical results were as good as, if not better than, those in the group that received multiple doses. If the results are as good, it would seem desirable to adopt a single-dose method, for the following reasons:

1. It is simpler, interferes less with the child's rest and sleep during the time when he is most ill and is less demanding on the nursing staff.

2. It decreases the incidence of the most common toxic effect, nausea and vomiting.

For the ordinary case of uncomplicated pneumonia it would appear that 0.3 Gm. of sulfapyridine per kilogram of body weight administered in one dose will give satisfactory results. Lower doses are not so consistent in giving levels in the blood above 4 mg. per hundred cubic centimeters. Although the lower levels gave satisfactory results, it would seem safer to use the larger dose. If it is thought desirable to maintain levels in the blood above 4 mg. per hundred cubic centimeters for more than twenty-four hours, the dose can be repeated at eighteen- to twenty-four-hour intervals. Such repetition is probably advisable when the patient cannot be watched closely. It will probably decrease the number of cases in which a secondary rise in temperature occurs.

After the oral administration of a single dose of 0.3 Gm. of sulfapyridine per kilogram of body weight, the free sulfapyridine in the blood usually reaches levels of at least 4 mg. per hundred cubic centimeters within a few hours. In most cases a considerable concentration persists for at least twenty-four hours.

In some cases the highest levels of the blood occur after twenty-four hours, indicating that absorption from the gastrointestinal tract may continue for that length of time.

The administration of a single dose of sulfapyridine of 0.3 Gm. per kilogram of body weight to children with pneumonia gives satisfactory therapeutic results.

Levels in the blood above 2 mg. per hundred cubic centimeters give satisfactory results in most cases of pneumonia in childhood.

The use of the single dose method simplifies the treatment of pneumonia and decreases the incidence of toxic effects.

Item

Announcement will shortly be made of unassembled examinations to be given for the following positions in the West Virginia State Health Department:

Director, Hygienic Laboratory	\$320-375
Senior Bacteriologist	150-200
Senior Serologist	150-200

Residence in West Virginia has been waived in consideration of the applications for these positions. However, residents of this state may be given preference in making appointments. Complete information may be obtained by writing directly to the Merit System Council, Morgantown, W. Va.

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PROGRESS

ALLERGY OF THE ABDOMINAL ORGANS*

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ALTHOUGH it has long been recognized that abdominal organs may participate in allergic reactions, there have been relatively few reports dealing with this subject. Because of the inaccessibility of the abdominal organs, considerable speculation has arisen as to the seat of the allergic processes and the nature of these reactions.

My colleagues H. Straus, I. Gray, M. Harton, S. Livingston, and D. Grayzel, and I have attempted to reproduce and study allergic reactions of the abdominal organs under experimental conditions. Investigations, not feasible in human beings, were carried out in the rhesus monkey, in which it is possible to induce allergic reactions closely resembling those occurring in man. It is hoped that the brief analysis of these experimental findings presented herewith will facilitate a better understanding of allergic reactions occurring within the human abdomen.

The primary experimental principle applied in most of these investigations was the production of allergic reactions in tissues which had been passively sensitized locally with human serum, containing atopic reagin antibodies. These sera were obtained from allergic individuals who were clinically very sensitive to such allergens as cottonseed, peanut, fish, egg, etc., and who showed very strong cutaneous reactions when tested with weak extracts of these allergens. This cutaneous sensitivity could be transferred to the skin of normal individuals by an intracutaneous injection of 0.05 ml. of the reagin-containing serum. The allergic reaction at the sensitized site was elicited from twenty-four to forty-eight hours later by feeding the related antigen, i.e., peanut, cottonseed, fish, egg, etc., or by an intravenous injection of an extract of that antigen. Oral administration of the antigen was regularly followed, within from five to

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fifteen minutes, by the entrance of traces of the antigen into the circulation and by the formation of a wheal at the sensitized cutaneous site.¹ This reaction resulted from the union of the circulating antigen with the reagents, which had remained fixed in the skin at the site of sensitization. The intravenous injection of the antigen usually resulted in a more rapid onset and in a more intense reaction than that which occurred following enteral administration. Both methods of inducing the allergic reaction were employed, depending upon the needs of the experiment. In the earlier investigations sensitization was confined to the skin; in later studies, mucous membranes, other tissues, and even complete organs were successfully sensitized.

METHODS OF EXCITING ALLERGIC REACTIONS IN ABDOMINAL ORGANS

Under physiologic conditions offending allergens may reach and excite abdominal organs in two ways. Any specific excitant introduced into the alimentary tract may, by direct contact, induce allergic reactions in the organs through which it passes. It is also possible for any antigen which gains entrance into the blood stream to reach sensitive abdominal organs by this route. The most natural way for antigens to reach the circulation is by way of the alimentary tract. In our studies on normal individuals, fish, egg, nut, cottonseed, mustard, milk, and other antigens almost routinely appeared in the blood stream within from two to thirty minutes after their ingestion.¹ This rapid absorption of the allergen accounts for many of the explosive allergic reactions which have been seen to follow, almost immediately, the ingestion of offending allergens. These reactions may affect widely separated organs, including parts of the digestive system distant from the site of administration and absorption of the excitant. That any one of several closely related organs or only one type of tissue may participate in an allergic reaction, while others in the same individual appear to remain unaffected, is recognized as a common occurrence in allergy, although an adequate explanation for this phenomenon is still lacking.

Passage of allergens into the circulation has been demonstrated to occur almost regularly from all parts of the alimentary tract, including the esophagus, stomach,² duodenum,³ ileum, colon,⁴ and rectum.⁵ Gastric acidity is an important factor in influencing the amount and rapidity of allergenic absorption. Gastric hyperacidity definitely retards and diminishes absorption, not only from the stomach and duodenum but also from the rectum.⁵ Hypoacidity and anacidity produce the opposite effects. Absorption of allergens may also be delayed or prevented by the previous oral administration of other food, oil, kaolin, pepsin, hydrochloric acid, or similar substances.⁶ Alcohol promotes and accelerates absorption. The importance of these experimental findings in the management of food allergies is obvious.

Allergen, administered rectally, may enter the blood stream as rapidly as that introduced into the duodenum, and even more rapidly than that taken by mouth.⁵ It should also be remembered that other mucous membranes and lining surfaces permit the rapid passage of antigens. Hence, sensitive abdominal organs may be specifically excited by allergens which have been absorbed by way of the upper and lower respiratory tract^{7, 8} and through the vaginal and cervical

mucous membranes.⁹ The same may be said in relation to allergens which have been introduced into the pleural, pericardial, and peritoneal cavities,¹⁰ and into the urinary bladder¹⁰ and the gall bladder.⁴ Similar effects may also occur when antigen is rubbed into the skin¹¹ or administered by the intraspinal,¹⁰ intramuscular, intracutaneous, or subcutaneous routes.

Minute quantities of antigen, containing as little as 0.0001 mg. of nitrogen, when given intravenously, have proved sufficient to induce maximal allergic reactions in passively sensitized tissue.⁵ The severity of allergic symptoms may, therefore, be far more intense than might be expected from relatively slight contact with the excitant. Moreover, it seems likely that in allergic incidents involving abdominal as well as other shock organs, observers are apt to overlook the importance of less obvious contacts with excitants, such as those occurring by inhalation and by vaginal, rectal, or parenteral administration.

ALLERGIC REACTION IN PASSIVELY SENSITIZED INTESTINAL MUCOUS MEMBRANE IN HUMAN BEINGS

An insight into the nature of the allergic reaction in the human alimentary mucous membranes was obtained by studies of the experimental allergic reaction induced in the sensitized mucous membrane of the rectum.¹² In this study the antigen was administered either rectally or orally. The reaction at the sensitized mucous membrane site appeared in from five to twenty minutes.

Edema, hyperemia, and hypersecretion were the cardinal characteristics of the reaction developing at the sensitized mucous membrane site. Edema appeared first, associated with a pallor of the mucous membrane which was superseded, within a few minutes, by marked hyperemia. Hypersecretion of mucus occurred over the affected area. The entire reaction reached its peak within fifteen minutes and then began to recede rapidly, disappearing almost completely in about an hour. The subjective symptoms varied with the site of sensitization. Pruritus, rectal fullness, and burning, as well as a desire to empty the bowel, were experienced in variable degree, depending on the site of the reaction. The closer the reaction to the anus, the more varied and intense were the symptoms. Few subjective symptoms were noted when the distance from the anal orifice was as much as 6 cm.

To observe the reactions in the ileum and colon mucous membranes, two patients, one with an ileostomy, the other with an ileocolostomy, were studied.⁴ Sites on the exposed mucous membranes were sensitized by intramucosal injections of reagin-bearing serum. Within four or five minutes following oral administration of the antigen, the allergic reaction started. Pallor and hypersecretion of the sensitized area were followed, in a few minutes, by edema which became progressively greater for about half an hour (Fig. 1). The pallor was replaced by marked hyperemia and the secretion of mucus became profuse. The entire reaction receded at the end of an hour. Except for itching or burning of the surrounding skin, no subjective symptoms accompanied these reactions.

Direct application of antigen, on an applicator, to a sensitized mucous membrane site on the rectum¹² or colon,⁴ produced an allergic reaction of moderate intensity in about four or five minutes. As a result of this application, sufficient antigen was absorbed to excite a previously sensitized cutaneous site. At

this time, the mucous membrane sites began to react maximally. This sequence of events, noted repeatedly in these and other experiments, again emphasizes the importance of blood-borne antigen in the elicitation of maximal allergic responses. Although direct contact of mucous membranes with offending allergens may produce severe reactions, it seems likely that, in most instances, reactions of maximal intensity are caused by blood-borne excitants. It is of interest to note that, in the above experiments, the antigen, which was gently applied on an applicator to the sensitized mucous membrane site (without rubbing), reached the circulation by passing through the sensitized area itself. Sensitive mucous membrane, therefore, is not a barrier to the absorption of offending allergens.

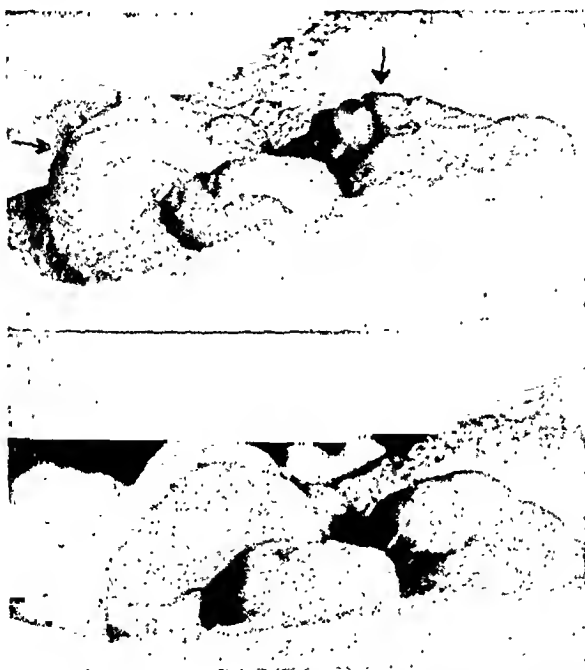


Fig. 1.—Above, Human ileum (left) and colon (right) at onset of allergic reaction. Below, Human ileum (left) and colon (right) after thirty minutes, at height of allergic reaction. Arrows mark sites of sensitization.

ALLERGIC REACTIONS IN PASSIVELY SENSITIZED ORGANS IN THE MONKEY

In the monkey sensitization of mucous membrane was accomplished with laparotomy under sterile precautions, by the injection of sensitizing serum into mucous membranes exposed by incisions into various organs.¹³ One or more sites were sensitized in areas as far away as possible from the neighborhood of the incision into the organ. At times intussusception of one organ into another which had been opened permitted sensitization of the first without actual incision into it. Either the oral or the intravenous method of excitation of the allergic reaction was employed.

In studies on the passively sensitized stomach¹³ in the monkey, the most pronounced changes occurred in the uncut organ in which the mucous membrane had been sensitized at several sites, through an opening in the duodenum, near the pylorus. The administration, by stomach tube, of the meal containing the

antigen resulted in the onset of the allergic reaction within three or four minutes. At first the walls of the organ became pale and turgid. Peristaltic waves increased in frequency and intensity. Prepyloric and pyloric spasm developed and persisted for long periods of time. The size of the organ became definitely diminished. On section the mucous membrane was seen to be markedly hyperemic and edematous. This was particularly noticeable at the pylorus, which was almost closed by thickening of the mucous membrane on the gastric side, while the lining of the unsensitized duodenum remained normal. Mucus flowed excessively from the gastric mucous membrane.



Fig. 2.—Opened gall bladder in the monkey showing allergic reaction involving the lower one-half of the organ

In the cecum and colon, in which single sites of the mucous membranes had been sensitized, similar reactions developed.¹³ With the onset of the allergic reaction, blanching of the affected gut developed, followed in a short time by local swelling and edema. Hyperperistalsis became noticeable in this region of the bowel and, in some instances, definite tonic contractions, lasting for five or ten minutes at a time, were noted at the sensitized area. On section of the bowel, the mucous membrane revealed the usual picture of hyperemia, marked edema, and hypersecretion.

Microscopic examination¹⁴ of tissue taken from the site of the allergic reaction showed the lining cells of the mucosa to be only slightly involved. The submucosa in particular, and, to some extent, the serosa were edematous. Their vessels were distended and showed margination of the white blood cells. Around the vessels and throughout the tissues were infiltrations of eosinophiles, lymphocytes, large mononuclear and some polymorphonuclear leucocytes.

In experiments on the gall bladder¹⁵ incision into the organ was avoided. Sensitization was effected by injecting the sensitizing serum directly into the wall of the organ at several sites. The allergic reaction was induced by intravenous injection of the antigen. Within a minute or two that part of the organ which had been sensitized grew pale and the surface vessels became more prominent. This part of the wall became edematous and appeared to bulge and throb. Spasm was not observed in any part of the organ in any of these experiments.

On section of the gall bladder at the height of the reaction the wall of the involved area appeared several times thicker than that of the unaffected part. As in the other organs, edema, hyperemia, hypersecretion, and loss of surface markings characterized the allergic mucous membrane (Fig. 2).

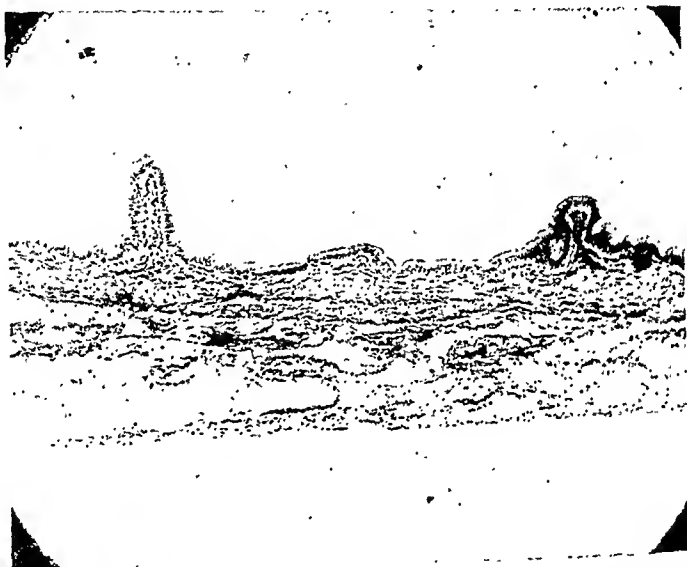


Fig. 3A.—Microscopic picture of normal part of the gall bladder in the monkey.

Microscopic examination of the allergic gall bladder wall revealed the mucosal epithelium to be intact and only slightly affected (Fig. 3). The lamina propria was markedly edematous. The blood vessels were engorged and showed some margination of white blood cells. There was a pronounced cellular infiltration consisting of eosinophiles, lymphocytes, large mononuclear cells, and some polymorphonuclear leucocytes. The edema invaded the other layers of the gall bladder, spreading between the muscle bundles and fibers. Vascular congestion and cellular infiltration also were present in these areas but not to the same degree as in the lamina propria. The nonsensitized part of the organ showed none of these changes on microscopic examination.

ALLERGIC REACTIONS IN THE ALIMENTARY TRACT OF FOOD-SENSITIVE CHILDREN

Special roentgenographic studies in food-sensitive children, made by Fries and Zismor,¹⁶ rendered it possible to visualize, to some extent, the behavior of the human alimentary tract during allergic reactions. Thirty children, who were known to manifest gastrointestinal disturbances following the ingestion of certain foods, were first studied by fluoroscopic and roentgenographic observa-

tions following the ingestion of a standard barium meal. About a week later a similar meal, to which, without the patient's knowledge, a small amount of an offending allergen had been added, was administered under the same experimental conditions. The x-ray findings were then compared with those of the control series.

Some characteristic changes were found in many of these children, following the ingestion of the allergen-barium meal. In most instances a pronounced gastric retention developed. The stomach appeared hypomotile and hypotonic. The duodenal bulb, in many cases, seemed to fill with difficulty. At times barium was still observed in the stomach six hours after the allergen-barium meal, whereas the control series had shown complete emptying of the stomach in a much shorter time.



Fig. 3B.—Microscopic picture of gall bladder wall involved in allergic reaction.

The progress of the allergen-barium meal through the small bowel varied with the patient. In some its passage was definitely accelerated, while in others no unusual feature was detectable in its progress. In the large bowel evidences of spasm or dilatation were frequently observed. This finding was not constant and, whenever present, it did not affect all portions of the bowel equally.

The administration of allergen-barium enemas to the allergic children usually precipitated spasm in some parts of the large bowel. The seat of the tonic contractions varied with the patient, although the transverse colon seemed to be affected most frequently. In some dilatation of the bowel resulted from this contact with the offending allergen.

The oral administration of the allergen-barium meal in 30 food-sensitive children produced subjective symptoms in 73 per cent.¹⁷ Nausea was noted most frequently, occurring in 43 per cent. Vomiting, usually within several minutes after administration of the meal, occurred in 23 per cent. In 20 per cent abdominal pain was pronounced. This pain had no characteristic localization, but

was most frequently reported to be in the center of the abdomen. Burning or itching of the mouth or throat was a complaint of only 7 per cent.

Abdominal pain was a more frequent and severe symptom among the 16 children who received allergen-barium enemas. Seventy-five per cent of these experienced severe abdominal pain. Nausea occurred in only 6 per cent of this series, but tenesmus was a complaint of 24 per cent.

ALLERGIC SHOCK TISSUE

The foregoing findings suggest apparent similarities in the allergic reactions of the gastrointestinal tracts in man and in the passively sensitized rhesus monkey. In both, edema, hyperemia, and hypersecretion characterize the allergic mucous membranes. In both, the emptying time of the stomach is delayed, following the ingestion of the offending allergen. Spasm of the small and large bowels may occur in both, although it is not so pronounced or so regular a finding in man as it is in the monkey. Dilatation of the bowel was observed more frequently in man.

These observations render it necessary to reopen the question as to whether the smooth muscle is the seat of the allergic reaction in man and plays the same role as shock tissue that it does in the anaphylactic reaction of the guinea pig, rabbit, and dog. This assumption, accepted as fact in some quarters, finds no support in the above findings. Smooth muscle spasm, which characterizes the anaphylactic reaction in the guinea pig, is, by no means, a constant manifestation of the allergic reaction of the human bowel. Moreover, on testing intestinal smooth muscle of the passively sensitized monkey by the Schultz-Dale technique, Albert and I¹⁸ found no evidence of sensitivity of this tissue. Negative results were obtained with these intestinal strips, despite the fact that these animals had been passively sensitized with large doses of human reagin-bearing sera of high titer, which easily produced sensitization of the skin and mucous membrane of the same animals. Even when anaphylactic (precipitin) antibodies were employed for passive sensitization of the rhesus monkey, no evidence of sensitivity could be found in the smooth muscle of intestinal strips.¹⁹ Negative results were also obtained in monkeys in which active sensitization was attempted using the technique suggested by Kopeloff, Davidoff, and Kopeloff.²⁰

In man, the only smooth muscle studies reported are those of Tuft,²¹ who tested uterine muscle strips obtained during the cesarean section of a woman with precipitins and reagins to horse serum in her blood. Tests with horse serum, by the Schultz-Dale technique, failed to produce any specific contractions in the uterine muscle. It, therefore, seems unlikely that, in man as well as in the monkey, smooth muscle is the shock tissue. This viewpoint finds further support in the histologic studies of the allergic reactions already described. It will be recalled that, although edema, vascular dilatation, and cellular infiltration involved the connective tissue around the muscle bundles and between the muscle fibers, the latter did not seem to share in the inflammatory process.

It also seems unlikely that the epithelial cells of the mucous membranes are the seat of the allergic reactions. These cells did not appear to be seriously altered in the histologic studies of the allergic tissues. Moreover, the

topical application of an allergen to the surface of the sensitized rectal or intestinal mucous membrane did not elicit a maximal allergic reaction, an experimental finding similar to that which had been noted in connection with the passively sensitized nasal mucous membrane.²² The fact that blood-borne antigen, even though diluted, was able to elicit more intense allergic responses than concentrated antigen applied directly to the epithelial surface tends to minimize the importance of the latter as a shock tissue.

OTHER ABDOMINAL ORGANS AND TISSUES SHARING IN ALLERGIC REACTIONS

In the histologic studies of the allergic abdominal organs, it was repeatedly noted that the peritoneum shared in the inflammatory reactions. When the sensitizing sera were introduced into the serosa of the intestine, the allergic reaction, subsequently elicited, was most pronounced in the peritoneal layer.¹³ The edema, in such an instance, tended to surround the gut and to affect the muscular and mucosal layers only to a slight degree. Frequently, the edema spread between the layers of the mesentery, increasing its thickness considerably. An increase of secretion was definitely noticeable over the affected serosa. It was also of interest to note that, when the peritoneum was the seat of the allergic reaction, tonic spasm of the bowel in this region was not commonly observed as it had been when the mucous membranes had been sensitized. All these findings suggest that the peritoneum itself may be the primary seat of an allergic reaction and that allergic peritonitis should be recognized as a possible cause of abdominal disturbances in allergic individuals.

Allergic cholecystitis is another clinical possibility which is not being given sufficient consideration in the differential diagnosis of pain in the right upper quadrant in allergic individuals. Experiments in the monkey, demonstrating that this organ can be sensitized, and the pathology of the allergic reaction occurring in the gall bladder have already been presented.¹³ Allergic involvement of the gall bladder may possibly account for the frequent failure to find roentgenographic evidence of gall bladder disease in allergic patients with the typical symptoms of cholecystitis. Moreover, the transient nature of the inflammatory process in allergy may account for the absence of pathology in the gall bladder by the time these patients reach the operating table after a protracted period of observation and preparation. The diagnosis, however, may be established by the fact that the feeding of the specific allergen induces gall bladder symptoms at will, while its elimination from the diet produces a complete cessation of symptoms.

Attempts to demonstrate sensitization of other abdominal organs and tissues have met with only partial success.¹³ The allergic reaction in the sensitized spleen in the monkey was a brief one. Following intravenous administration of the offending antigen, the spleen swelled rapidly and assumed a darker color. The process was only short-lived, however, and the organ returned to its normal state within a few minutes.

The allergic reaction in the passively sensitized uterus¹³ appeared, for the most part, to involve the peritoneum and subperitoneal connective tissue. The uterine muscle and endometrium seemed hardly to share in the edema.

Injection of the reagin-bearing serum into a lobe of the liver did not induce sensitization of hepatic tissue, and no evidence of an allergic reaction in this organ has thus far been obtained.¹³ Attempts at sensitization of renal tissue have invariably resulted in death of the animal before the experiment could be completed.

DISCUSSION

Although the reagin mechanism has been used to reproduce experimentally the allergic reactions in the various abdominal tissues and organs, it is not my intention to suggest that all allergic reactions are mediated by this antibody. It is, in fact, common knowledge that food hypersensitiveness occurs in the absence of reagins and, therefore, of positive cutaneous reactions to the offending allergen. In such instances, the clinician is compelled to rely on clinical trials and diets for the diagnosis. A close scrutiny of the patient's daily food diary and analysis of his symptoms in relation to each article of his diet may be necessary for the detection of the offending allergen. Coca²³ has recently suggested pulse rate studies as a means of diagnosis in nonreaginic food allergies.

Regardless of the absence of reagins in many forms of food hypersensitiveness, their use in the above investigations has yielded some definite experimental information as to the nature of the processes involved in abdominal allergy and opens the way for still further investigation of this little explored field.

REFERENCES

1. Walzer, M.: Studies in Absorption of Undigested Proteins in Human Beings. I. A Simple Direct Method of Studying the Absorption of Undigested Protein, *J. Immunol.* 14: 143, 1927.
2. Harten, M., Gray, I., Livingston, S., and Walzer, M.: Absorption of Undigested Protein From the Stomach, Esophagus and Gall Bladder in the Rhesus Monkey, *Abstr. J. Allergy* 10: 478, 1939.
3. Gray, I., and Walzer, M.: Studies in Absorption of Undigested Proteins in Human Beings. VII. The Absorption of Unaltered Protein Introduced by Duodenal Tube Into the Abnormal Gastrointestinal Tract, *Am. J. Digest. Dis.* 5: 345, 1938.
4. Gray, I., Harten, M., and Walzer, M.: Studies in Mucous Membrane Hypersensitiveness. IV. The Allergic Reaction in the Passively Sensitized Mucous Membrane of the Ileum and Colon in Humans, *Ann. Int. Med.* 13: 2050, 1940.
5. Gray, I., and Walzer, M.: Studies in Absorption of Undigested Protein in Human Beings. VIII. Absorption From the Rectum and a Comparative Study of Absorption Following Oral, Duodenal and Rectal Administrations, *J. Allergy* 11: 245, 1940.
6. Walzer, A.: Studies in Urticaria. V. The Effect of the Oral Administration of Certain Substances Upon the Experimental Wheal of Alimentary Origin. *Deliberationes Congressus Dermatologorum Internationalis*, IX 2: 134, Sept., 1935.
7. Cohen, M. B., Ecker, E. E., Breitbart, J. R., and Rudolph, J. A.: The Rate of Absorption of Ragweed Pollen Material From the Nose, *J. Immunol.* 18: 419, 1936.
8. Sulzberger, M. B., and Vaughan, W. T.: Experiments in Silk Hypersensitivity and the Inhalation of Allergen in Atopic Dermatitis (Neurodermatitis Disseminatus), *J. Allergy* 5: 554, 1934.
9. Rosenzweig, M., and Walzer, M.: The Absorption of Antigen From the Vagina and Cervix in Humans, *Abstr., J. Allergy* 9: 395, 1938.
10. Unpublished experiments.
11. Walzer, A.: Cutaneous Absorption. I. A Direct Technic for Demonstrating the Percutaneous Absorption of Antigens, *Arch. Dermat. & Syph.* 41: 692, 1940.
12. Gray, I., and Walzer, M.: Studies in Mucous Membrane Hypersensitiveness. III. The Allergic Reaction of the Passively Sensitized Rectal Mucous Membrane, *Am. J. Digest. Dis.* 4: 707, 1938.
13. Walzer, M., Gray, I., Strans, H. W., and Livingston, S.: Studies in Experimental Hypersensitiveness in the Rhesus Monkey. IV. The Allergic Reaction in Passively Locally Sensitized Abdominal Organs, *Preliminary Report, J. Immunol.* 34: 91, 1938.

14. Grayzel, D., and Walzer, M.: The Pathology of the Allergic Reactions in the Passively Sensitized Tissues of the Macacus Rhesus Monkey, Abstr., *J. Allergy* 10: 478, 1939.
15. Walzer, M., Gray, I., Harten, M., and Livingston, S.: The Allergic Reaction of the Passively Locally Sensitized Gall Bladder in the Rhesus Monkey, Abstr., *J. Allergy* 10: 70, 1938.
16. Fries, J. H., and Zizmor, J.: Roentgen Studies of Children With Alimentary Disturbances Due to Food Allergy, *Am. J. Dis. Child.* 54: 1239, 1937.
17. Fries, J. H., and Zizmor, J.: Gastrointestinal Allergy in Children, *J. Pediat.* 16: 69, 1940.
18. Albert, M., and Walzer, M.: Schultz-Dale Studies in the Rhesus Monkey Passively Sensitized With Human Reaginic Sera, Abstr., *J. Allergy* 10: 71, 1938.
19. Albert, M.: Studies in Anaphylaxis in the Rhesus Monkey, Abstr., *J. Allergy* 9: 302, 1938.
20. Kopeloff, N., Davidoff, L. M., and Kopeloff, L. M. General and Cerebral Anaphylaxis in the Monkey (*Macacus Rhesus*), *J. Immunol.* 30: 477, 1936.
21. Tuft, L.: Active Sensitization of Human Uterine Muscle, Preliminary Report of Experimental Attempts, Abstr., *J. Allergy* 9: 390, 1938.
22. Sherman, H., Kaplan, C., and Walzer, M.: Studies in Mucous Membrane Hypersensitivity. II. Passive Local Sensitization of the Nasal Mucous Membrane, *J. Allergy* 9: 1, 1937.
23. Coca, A. F.: Familial Nonreaginic Food Allergy as a Predisposing Cause of Common Cold, *J. Lab. & Clin. Med.* 26: 757, 1941.

CLINICAL AND EXPERIMENTAL

FAMILIAL NONREAGINIC FOOD ALLERGY*

ITS SPECIFIC DIAGNOSIS AND TREATMENT

ARTHUR F. COCA, M.D., ORADELL, N. J.

IN TWO previous informal publications^{1, 2} it was stated that certain allergic clinical reactions to foods were so constantly accompanied by tachycardia that in all the few cases that had been observed the offending foods could be identified through the use of that objective criterion.

This observation seemed especially welcome because the usual cutaneous tests for food sensitivity failed in nearly all the cases studied—as they have in nearly all the 19 succeeding ones that were so tested.

The findings were reported informally because the cases seemed too few to be acceptable in the usual media of publication, and yet interesting enough to suggest a further trial of the method of diagnosis in suitable cases.

More recently, additional cases have become available for study, and it is now evident that another category of familial allergic disease must be recognized as quite distinct from the previously recognized atopic group of bronchial asthma, hay fever, and atopic dermatitis. Briefly, the separation of the two categories is required on account of (1) differences in symptomatology; (2) difference in mechanism; and (3) difference in the hereditary factor. Only the first two differences will be discussed in the present report. However, the two terms that have been tentatively chosen to designate the two categories will be used for convenience in this paper. These are *atopy* (reaginic asthma, hay fever, atopic dermatitis) and *familial nonreaginic food allergy* (urticaria, allergic headache—migraine, gastrointestinal food allergy, and a number of other more or less serious clinical manifestations).

This investigation has already taken several different directions, detailed mention of all of which cannot be made in a single report of this kind. It is intended, in this first paper, to present evidence to indicate: (1) that specific tachycardia is a reliable criterion of nonreaginic food-allergic reaction; (2) that this criterion can be practically employed with a high percentage of success in the complete relief of food-allergic symptoms.

The eight charts presented are examples of transient tachycardia in some of which there is a close association with the ingestion of a particular food and with one or more clinical symptoms of food allergy.

In these graphic records of the pulse rate in four of the 31 persons who have been relieved of their food-allergic symptoms under the regime of trial diet that

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has been described elsewhere,^{1, 2} three characteristic phenomena are illustrated: (1) the rapidly alternating tachycardia and remission of the high pulse rate; (2) the latent period of the food-allergic reaction, and (3) the recurrent reaction.

The patient referred to in Chart 1 is a particularly interesting object of study because she was subject to paternally inherited atopic asthma and hay fever (pollen, house dust, wheat), as well as to the nonreaginic food allergy which she inherited from her mother. Ingestion of wheat, especially possum, causes an attack of asthma but no tachycardia, and her serum passively sensitizes the normal human skin, markedly to house dust and distinctly, though not markedly, to wheat. On the other hand, potato, pork, orange, and coffee, all cause tachycardia, headache, and other clinical symptoms of nonreaginic food allergy (Table I), but the patient's serum does not passively sensitize the normal human skin to these four foods.

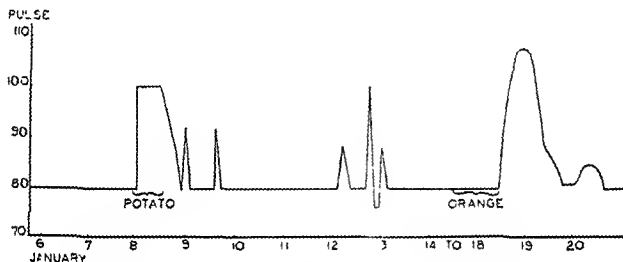


Chart 1.—The course of the pulse rate in patient M. M. D. on the sixth to the twentieth day of observation.

This demonstrated coincidental occurrence of the reaginic atopy and the nonreaginic food allergy in the same person suggests the possibility that the two forms occasionally may be exhibited by the same person to the same food. Hence, it will not be necessary, if, in the blood of a patient with migraine (for example), atopic reagins should be demonstrated that are specific for an excitant of it, to conclude that the reagins in that instance represent the specific mechanism of the migrainous attack.

The following additional features of this case are of interest:

(1) Beef, puffed wheat, or bread, milk, and twice ultrafiltered lemon juice were taken throughout the period indicated in Chart 1.

(2) Although potato was discontinued after lunch on the eighth day, the pulse rate rose twice on the ninth day. This recurrence of symptoms after discontinuance of an allergenic food is also illustrated in the record of the pulse rate after the ingestion of orange by this patient. There was mild recurrence of tachycardia and also symptoms (headache) on the second day after the discontinuance of orange.

(3) Orange was taken in liberal quantity at all three meals for nearly two days before symptoms appeared. This was the first observed instance in this study of the "latent period," which presently will be discussed more fully.

(4) The causes of the brief but considerable acceleration of the pulse rate on the twelfth day were not discovered. There may have been an accidental addition of some unsuspected allergen in the diet on that day.

(5) The level line at 80 indicates the highest recorded pulse rate (observed before and after each meal) during the indicated period. The pulse rate was frequently lower—sometimes as low as 72.

(6) Tachycardia was sometimes present for an appreciable time after the other symptoms had disappeared. In another person noticeable tachycardia has frequently been absent when the patient was suffering severe heartburn due to ingestion of relatively small quantities of an allergen.

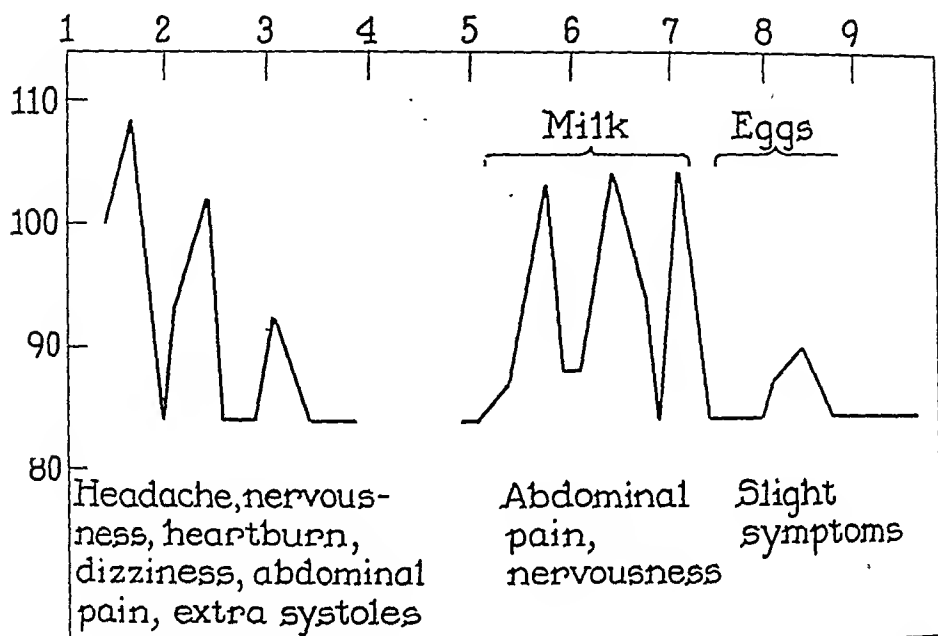


Chart 2.—The course of the pulse rate in patient A. R. on three days previous to the period of trial diet, and on the first five days of the trial diet.

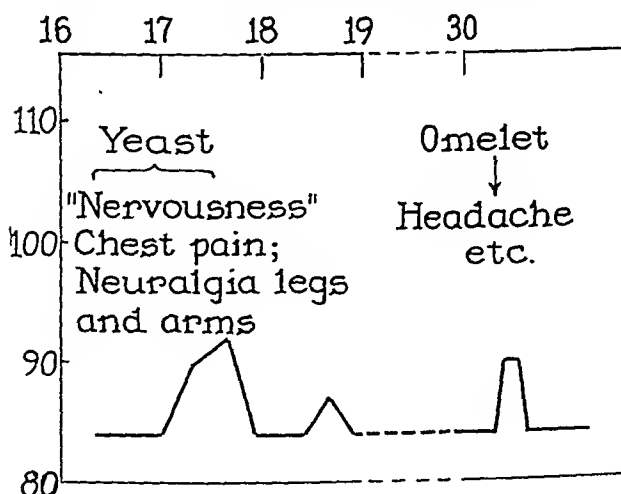


Chart 3.—The course of the pulse rate in patient A. R. on the sixteenth to the thirtieth days of observation.

A young biological chemist, A. R., whose pulse record is illustrated in Charts 2 and 3, had since February, 1939, been suffering from the symptoms mentioned in Chart 2 and had lost 25 pounds in weight. The pulse record shown under the figures 1 to 4, representing the first four days, shows the range of the pulse rate over 84 (which was found later to be his normal maximum) on his usual diet, which included generous amounts of milk and bread. On the fifth and sixth days, and on the morning of the seventh day, he took only milk every hour, and became convinced, at the end of this trial, that milk does not agree with him. He changed to a pure

egg diet from lunch on the seventh day and his symptoms lessened, the pulse rate on the eighth day showing a slight recurrent reaction to milk. After the ninth-day period he added wheat, beet sugar, and pork to his diet without experiencing symptoms, his pulse remaining under 84.

Following the ingestion of a yeast-raised zwieback, his symptoms recurred with a mild tachycardia. On the sixteenth and seventeenth days, he took several cakes of Fleischmann's yeast and, after a latent period of about a half day, experienced clinical symptoms with tachycardia. On the eighteenth day his pulse registered a slight, recurrent reaction. During the period between the nineteenth and thirtieth days, in which other foods were added to the diet, he was symptom free. The thirtieth day he spent in New York City where, as he reported, he "became overexcited and fatigued by climbing subway and elevated steps"; he ascribed to this unusual experience the headache and tachycardia recorded on that day. However, inquiry revealed that at lunch he had eaten omelet, not being aware of the milk content of this dish. He has been symptom free during the succeeding eighteen months and has gained 35 pounds in weight.

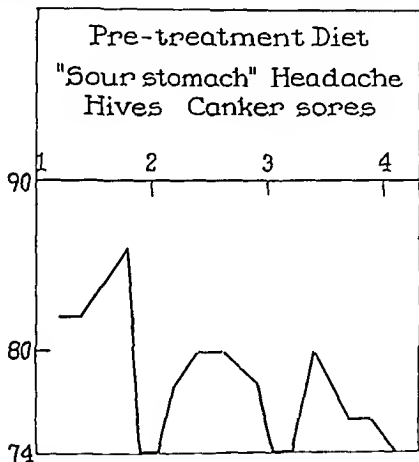


Chart 4.—The course of the pulse rate in patient J. G. on three days prior to the period of trial diet.

Trials with 30 different foods have revealed no other sensitivities.

The serum of this patient does not cause the slightest detectable passive sensitization of the human skin to milk.

J. G., a 36-year-old man, active in the management of a large city gauge, complained chiefly of a headache on the left side which recurred at one- to two-week intervals and incapacitated him for about three days. The recurrent headaches were of eighteen years' duration, and the most serious step in his previous treatment of them had been an exploratory abdominal operation performed at his insistence. Charts 4 and 5 show the course of his pulse rate in the first three days on a general diet, and in the first five days of the "trial diet."

It is seen in Chart 4 that his maximal pulse rate in the three days previous to the beginning of treatment was not very high—86. However, on an exclusive diet of beef on the fifth day, and of beef and milk on the sixth day (Chart 5), the pulse rate did not pass above 74. Following the addition of wheat on the seventh day the maximal rate reached 90, and he had a headache. He took no wheat after the evening meal of the seventh day, and there

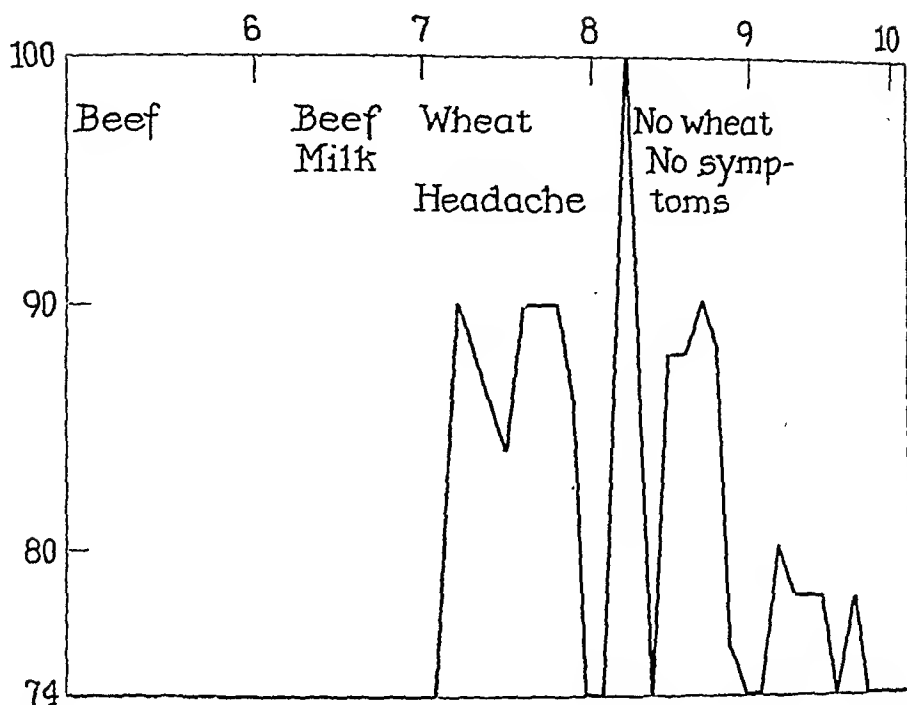


Chart 5.—The course of the pulse rate in patient J. G. on the first five days of the trial diet.

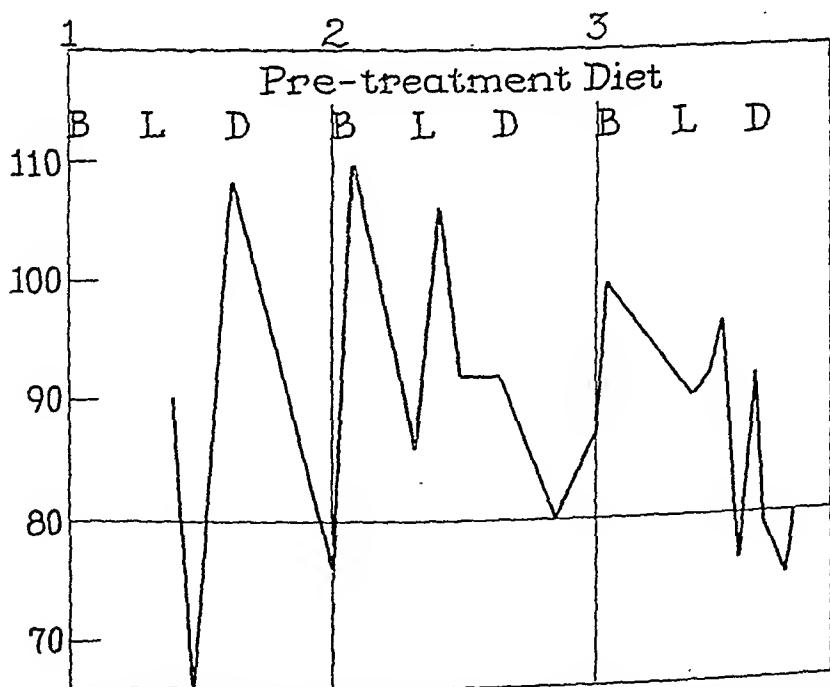


Chart 6.—The course of the pulse rate in patient C. T. on three days prior to the period of trial diet. B, breakfast; L, lunch; D, dinner.

were no symptoms thereafter. There was a vigorous recurrent tachycardia on the eighth day and a milder one on the ninth. The subsequent trial of coffee caused hives, but no headache. Cane sugar caused headache without hives. This patient has made trials of at least twenty-four other foods, with no unexplained clinical symptoms nor tachycardia in the succeeding twelve months.

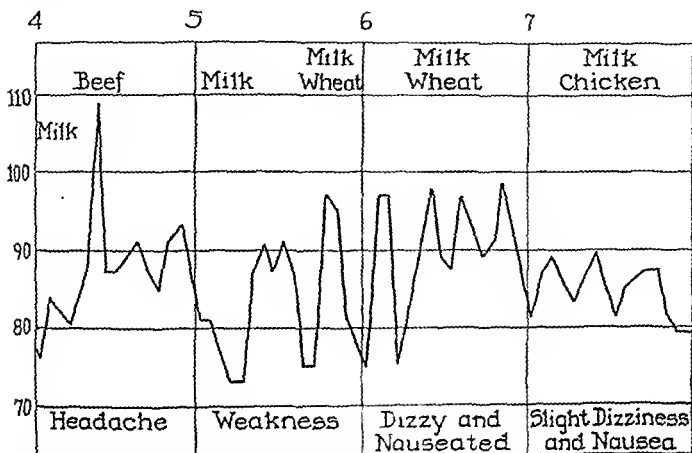


Chart 7.—The course of the pulse rate in patient C. T. on the first four days of the trial diet.

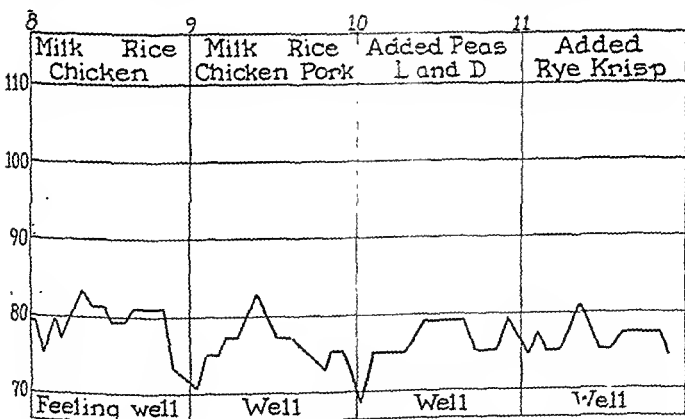


Chart 8.—The course of the pulse rate in patient C. T. on the second four-day period of the trial diet.

The cutaneous tests with the common inhalants and foods, including wheat and coffee (scratch tests with strong glycerinated extracts), were negative.

The record of the pulse rate in a young woman, C. T., employed by a large life insurance company in New York City, is shown in Charts 6, 7, and 8.

TABLE I

PATIENT	AGE	SYMPTOMS	RANGE OF PULSE RATE		TREATMENT BEGAN	ALLERGENIC FOODS
			BEFORE TREATMENT	AFTER TREATMENT		
E. F. C.	49	Migraine, mucous colitis, dizziness, neuralgia, nervousness, abdominal pain, physical tiredness, canker sores, intestinal bleeding, nausea, vomiting, "extrasyctoles," angina pectoris	Up to 180	68 to 84	Aug. 1935	Beef, lamb, fish, egg, milk, cereals, string beans, Lima beans, white potato, sweet potato, tomato, onion, artichoke, squash, cabbage family, lettuce, carrot, beet, spinach, turnip family, nuts, plum, pear, melon, pineapple, peach, banana, apricot, asparagus, parsnip, pepper, coffee, pork, orange
A. F. C.	65	Migraine, gastric pain (cramplike), abdominal pain, pain in region of gall bladder, heartburn, canker sores, severe dizziness, physical tiredness, gastrointestinal bleeding, extrasyctoles, psychic depression, nervousness, chronic rhinitis, constipation, neuralgia, conjunctivitis	56 to 100	58 to 72	Aug. 1935	Pork, fish, cereals, cane sugar, white potato, sweet potato, beet, carrot, spinach, peas, beans (string and Lima), cabbage family, lettuce, asparagus, parsnip, artichoke, turnip family, tomato, mushroom, maple sugar, chocolate, apple, olive, date, nuts, yeast, squash, pineapple, plum, peach, apricot, melon, orange, grapefruit, lemon, onions, grapes, pepper, coffee, tea, aluminum, fowl, cow's milk, banana
M. M. D.	27	Migraine, nausea, chest pain, marked physical tiredness, nervousness, vomiting, canker sores, constipation, bronchial asthma	70 to 100	70 to 80	Jan. 1936	Pork, potato, orange, coffee
A. R.	28	Migraine, nervousness, chest pain, heartburn, dizziness, extrasyctoles, neuralgia	66 to 108	70 to 84	Aug. 1939	Milk, yeast
G. T.	26	Nausea, diarrhea, neuralgia, extrasyctoles ("fluttery heart"), marked physical tiredness, canker sores, marked dizziness, psychic depression, constipation, "nervous and emotional instability—incurable"	65 to 112	72 to 78	Jan. 1940	Beef, cereals, cane sugar, orange, tomato, prune, grapefruit, potato, pineapple, onion, banana, strawberry, egg, lemon
J. G.	36	Incapacitating 3-day headaches at 1- to 2-week intervals; frequent urticaria and angioneurotic edema, heartburn	66 to 86	66 to 80	Jan. 1940	Wheat, sugar cane, coffee
H. E.	49	Dizziness, migraine, "gas," physical tiredness, anorexia	76 to 106	58 to 76	March 1940	Wheat, milk, coffee

W. W. F.	57	Headaches, heartburn, "indigestion," canker sores, marked physical tiredness, petit mal (frequent during past 20 years); epileptic sib	66 to 100	56 to 68	April 1940	Beef, orange, lamb
M. W. F.	52	Headaches, heartburn, "gas," indigestion, nervousness, marked physical tiredness	72 to 100	58 to 72	April 1940	Criseo, peanut, cinnamon, pecan
A. W. F.	17	Allergic sore throat	70 to 90	60 to 72	April 1940	Chocolate
F. C. F.	16	Severe headaches, dizziness, physical tiredness	58 to 84	58 to 70	April 1940	Chocolate, criseo
M. B.	22	Frequent spontaneous epistaxis	64 to 100	64 to 76	April 1940	Egg
P. W.	47	Constant severe headache, dizziness, marked physical tiredness, chronic rhinitis, post-nasal drip, bronchial asthma ("infectious")	68 to 100	62 to 78	April 1940	Beef, lamb, egg, cow's milk
R. M.	22	Frequent severe headaches, dizziness, nervousness, canker sores, "indigestion," constipation, marked dysmenorrhea	68 to 100	62 to 78	May 1940	Beef, egg, potato, tomato, chocolate, coffee
A. P.	70	Marked generalized urticaria (dollar size), chronic cough, constipation	72 to 100	70 to 74	May 1940	Beef, cow's milk, egg, yeast, olive, corn, banana, proprietary laxative
S. I. H.	40	Frequent headaches	68 to 100	64 to 80	June 1940	Beef, lamb, white potato, tomato, orange, yeast, sugar cane
H. A. S.	58	Marked urticaria at long intervals, occasional headaches, canker sores, neuralgia, physical tiredness	60 to 105	60 to 74	June 1940	Beef, bean, asparagus, chocolate, corn, Swiss chard, peas, cinnamon, eggplant, coffee
J. F.	53	Headaches, angioneurotic edema, physical tiredness, chronic rhinitis, postnasal drip of 10 years' duration, constipation	68 to 108	68 to 80	June 1940	Lamb, egg, chicken, potato, tomato, grapefruit, chocolate, coffee, tea, orange
W. S. C.	41	Headaches, "indigestion," gas, nervousness, marked physical tiredness	46 to 70	46 to 62	July 1940	Beef, potato, cow's milk, tobacco
J. V.	35	Headaches (almost continual, incapacitating), asthma (mild), physical tiredness, chronic rhinitis	44 to 78	48 to 64	July 1940	Cow's milk, wheat, carrot, tomato, lettuce, banana, melon, lemon, peanuts, egg, peach, cereals, cabbage family, bacon, ham, pear, peppermint

TABLE I—CONT'D

PATIENT	AGE	SYMPTOMS	RANGE OF PULSE RATE		TREATMENT BEGAN	ALLERGENIC FOODS
			BEFORE TREATMENT	AFTER TREATMENT		
E. B.	50	Constant headache, nervousness, neuralgia; "very irritable," marked physical tiredness	82 to 108	72 to 76	July 1940	Wheat, pork
Dr. R.	26	Severe incapacitating headaches, physical tiredness, chronic rhinitis, dizziness, neuralgia, heartburn	70 to 90	70 to 76	Aug. 1940	Milk (cow's), egg, orange, grapefruit
R. F.	34	Severe headache (left side), heartburn, neuralgia, physical tiredness, nervousness, chronic rhinitis	64 to 114	62 to 76	Sept. 1940	Fowl, milk (cow's), wheat, corn, nuts, plum (prune)
A. F.	10	Headaches, bronchial asthma	82 to 124	74 to 78	Oct. 1940	Milk, beef, pork, squash, grape, peas, lettuce, tomato, cabbage family, celery, orange, peanut, marshmallow
N. W.	38	Severe headaches, indigestion, heartburn, canker sores, dizziness, physical tiredness, neuralgia, chronic rhinitis, nervousness	64 to 86	62 to 76	Nov. 1940	Pork, potato, sweet potato, tomato, pear, beef, cabbage family, peas, string beans, mushrooms
Dr. I. P.	46	Severe gastric pain (suspected ulcer) operation contemplated	84 to 104	72 to 78	Jan. 1941	Diagnosis incomplete Cow's milk, major allergen (if there are others they are negligible)
L. H. B.	20	Marked headaches, dizziness, physical tiredness, occasional urticaria, acne, nausea, vomiting	60 to 90	58 to 70	Jan. 1941	Wheat, chocolate, peas, onion, string bean, squash, white and sweet potatoes, spinach, rice, chicken, peach, apricot, orange, banana, tomato, grapefruit, pineapple, cabbage, carrots, lettuce, beets
J. J. V.	38	Headaches (frequent), bronchial asthma, nervousness, physical tiredness, acne, angioneurotic edema	70 to 94	62 to 74	Jan. 1941	Cow's milk, tomato, onion, potato, lamb, Lima bean, fish, oyster, tobacco, sweet potato, coffee, chocolate, orange, chicken, apple, banana, cabbage, celery
C. B.	23	Chronic rhinitis, bronchial asthma	72 to 100	67 to 72	Jan. 1941	Wheat, egg, lettuce, cane sugar
K. S.	48	Migraine, physical tiredness, constipation, nervousness, neuralgia, extrasystoles	70 to 110	70 to 80	Jan. 1941	Fish, lamb, potato, peas, string beans, carrot (tomato not tested but avoided on account of earlier experiences)
M. D. B.	Over 50	Heartburn, migratory neuralgia, constipation, physical tiredness	62 to 90	52 to 60	April 1941	Milk, orange, lemon, carrot, beet, asparagus (spinach not tested but avoided)

The outstanding symptoms in her case were recurring attacks of marked dizziness (without headache), which so interfered with her work as to make her continued employment uncertain. The company physicians had once placed her under psychiatric observation in a sanatorium for three months and had reached a diagnosis of "emotional and nervous instability" which they considered incurable. She consulted me only to inquire why she was always decidedly worse immediately after injections of "cold vaccine." At that time, under the threat of losing her position, and because her life was otherwise intolerable, she was contemplating suicide.

Since it was imperative that she be kept from her work not longer than ten days, more rapid additions to the diet were allowed than is usually advisable. The headache caused by beef (taken three times) was the first severe headache in her experience.

The first rise of the pulse rate on the fifth day was interpreted as recurrent from the reaction to beef; the second rise on that day was thought probably to be due to wheat. The patient has been found sensitive to all other cereals and sugar cane, and to orange, grapefruit, tomato, coffee, onion, banana, pineapple, strawberry, lemon, and egg.

During the succeeding nineteen months (to date) she has, with few exceptions,* been free from the dizziness as well as all the other minor symptoms (canker sores, nausea, diarrhea, "fluttering heart," neuralgia, and marked lassitude). The exceptional episodes could all be referred to the unwitting eating of one or the other of the allergenic foods. On one occasion she ate at the evening meal only the meat of a fowl that had been stuffed with bread, and the next day she suffered a mild dizziness.

Direct intracutaneous tests with extracts of the incriminated foods gave negative results. Intracutaneously injected into a known receptive patient (E. D.) her serum in a quantity of 0.05 c.c. failed entirely to sensitize the skin passively to beef, wheat, orange, and coffee (no tests were done with the other allergenic foods).

Table I presents the essential data of the 31 persons with nonreaginic food allergy who have been successfully treated with the method of trial diet controlled through the pulse rate.† The following points may be emphasized.

The range of the food allergic pulse rate varies greatly in different persons, for example, J. G. and F. C. F. showed high rates of only 86 and 84, respectively. The range of the normal pulse rate varies greatly in different persons; the high count (68) of W. W. F. is the same as the low count of J. F.

In most cases the symptoms mentioned in Table I could be induced by eating one or the other of the allergenic foods identified by means of the tachycardia. Many of those symptoms have not been generally recognized as due to food sensitivity; especially, nervousness, dizziness, allergic sinusitis, physical tiredness ("no 'count feeling"—Vaughan) and irritability. *All of these symptoms disappeared in the respective patients after the pulse rate became normal following the elimination of the allergenic foods, and they were usually reproducible in all such persons by the mere eating of the forbidden foods.*

In one patient with food allergy (Dr. "C.") the tachycardia, although amply present (range of 64 to 84, once 90), could not be practically used in the detection of the offending foods. On the one hand, the single pulse rate of 90 was recorded on a day when the patient was "feeling quite well"; whereas, on one morning when he "awoke feeling as though an attack were impending," the pulse rate ranged between 72 and 76. However, this patient showed other peculiarities. Dr. "C.," with much difficulty using the Andresen method, has identified "meat, fish, chicken, eggs, wheat, chocolate, and coffee" as allergenic

*These will be discussed in a later publication.

†In each instance the pulse rates were noted by the patient, the figures representing the half-minute count multiplied by 2. No instructions were given the patients as to posture, etc., when the counts were being taken. This and other practical considerations will be discussed in detail in a later publication.

in his case. Yet, on one occasion after having eaten chicken liberally on three successive days (just after a four-day attack), he was "feeling quite well" on the third day—the day of the single pulse rate of ninety. By avoiding the above-mentioned foods, or eating them in the respective "latent period"—"occasionally, or once weekly"—he has reduced his attacks to "one severe one and one mild one in 5 months."

There are two possible explanations of this failure to arrive at complete relief of the allergic symptoms. (1) The patient, being a doctor of medicine was in the habit of taking certain commonly used drugs at the first premonition of an "attack." It has been seen in other cases that drugs may so affect the pulse rate as to interfere with the interpretation of the record. (2) This case was being studied long before the allergenic action of metals and essential oils had been recognized.*

There have been a few other "failures," all of which are similarly explainable.

Most of the foods that have been identified as excitants of nonreaginic food allergy in the 31 patients that have been under this kind of treatment have been common ones. Table II shows the list of these foods.

TABLE II

FOODS MOST COMMONLY INCRIMINATED AS EXCITANTS OF FAMILIAL NONREAGINIC FOOD ALLERGY IN 31 CASES

Wheat	11	White potato	8
Orange	11	Chocolate	8
Egg	11	Coffee	7
Beef	10	Sugar cane	6
Milk	10	Sweet potato	5
Tomato	9		

LATENT PERIOD IN NONREAGINIC FOOD ALLERGY

In the case of A. F. C. it had been noticed that after sensitivity to a number of vegetable foods had been unquestionably established in several separate tests, in all of which the allergenic food had been eaten in generous quantity several times over a period of one or more days, if the food was avoided for a long time it could always be eaten once, often more than once, with impunity. I began to generalize my thoughts about this recurring phenomenon after I came across the following statement by Vaughan^{3a}: "This, we shall see, is very characteristic of food allergy, that an allergenic food may be eaten at times with impunity, at other times with consequent symptoms."

The thought took a practical turn upon the following observation of J. G. (see Chart 5), who had been found sensitive to wheat. About two months after his first test with wheat, he ate, only at one meal, an unstinted quantity of bread after which he suffered no clinical symptoms and observed no rise of the pulse rate above his normal maximum.

The relatively short interval (two months) between the two tests with wheat suggested the question how short the interval can be made without the occurrence of a reaction. This question was studied in the case of A. F. C., by administering at six- or seven-day intervals a number of vegetables and fruits to all of which he had been proved sensitive. Some of these foods (peas, string beans, Lima beans, beet, date, raisin) had not been eaten for several weeks, some not for several years (asparagus, broccoli, alligator pear, celery, apple, yam, squash, endive, tomato, onion, peach, pear, prune, blueberry, raspberry, and cane sugar). With three exceptions no tachycardia or allergic symptom followed the first two ingestions of these foods.

Broccoli caused no symptoms on the first ingestion, but after the second, six days later, there was heartburn and an eight-point rise of the pulse rate from the normal maximum of

*To be discussed in a later communication.

72. Squash on the first eating caused no reaction, but one week later it caused nervousness and a tachycardia (93). Yam, eaten for the first time after an abstinence of many years, caused headache and heartburn, the latter being felt within a few hours, and the former on the following morning.

Patient A. R. (see Charts 2 and 3), after abstaining from milk for six months, has taken a glass of milk once without experiencing symptoms or tachycardia.

The latent period is illustrated in the case of M. M. D. (Chart 1), who ate oranges from dinner January 16 to lunch January 18 before headache and tachycardia (to 104) began.

There was a similar experience with oranges in the case of C. T., symptoms being delayed for nearly forty-eight hours.

In some instances in the case of A. F. C. opportunity has been had to observe that the longer the interval of abstinence from an allergenic food the longer the latent period. Thus, string beans, after having been previously avoided for a number of years, were eaten almost every day from October 3, 1939, until March 10, 1940, when symptoms and tachycardia appeared, which ceased about twenty-four hours after the last consumption of string beans. On April 13 eating of string beans was resumed, at first at four- to five-day intervals and from May 22 at seven-day intervals without symptoms until June 19, when mild headache appeared in the night, and again June 26, when abdominal pain followed soon after eating the beans. After an interval of two weeks, indulgence in a liberal quantity of the beans was followed shortly by slight intestinal discomfort. The pulse rate was 72 after walking up two flights of stairs.

Grapes and raisins had been previously avoided for about nine years, when after January 10, 1940, they were taken almost daily without symptoms, until February 12, on which date heartburn, flatus, and headache appeared. Lettuce, which was also being eaten, was discontinued on February 16, but the headaches, heartburn, and flatus continued until twenty-four hours after grapes and raisins were omitted on February 28. There were no allergic symptoms after February 29 until March 4, heartburn from rice wafers) and March 10 when headache marked the return of sensitivity to string bean.

From the foregoing data it is seen that the latent period of nonreaginic food allergy is not constant. It varies in different persons and in the same individual with respect to different foods. The latent period has been encountered in 13 of the 31 patients (E. F. C., M. M. D., C. T., J. G., W. W. F., R. M., H. A. S., J. F., W. S. C., A. R., R. F., M. D. B., I. H. B.). Most of the 13 patients are making regular practical use of the phenomenon by eating the respective allergenic foods at safe intervals.

The phenomenon of the latent period is clearly described by Vaughan,^{2a} who cites in illustration a case of sensitivity to egg with avoidance of that food "for three years." The patient, being found "negative" to the cutaneous test, resumed the eating of egg and, one month later, began again to exhibit the allergic symptom (migraine). In such a case Vaughan speaks of the allergenic food as a "build-up" food, referring to the time required, after the period of avoidance, for the re-establishment ("build up") of the sensitivity.

SIGNIFICANCE OF THE LATENT PERIOD OF NONREAGINIC FOOD ALLERGY

An interpretation of the latent period of food allergy is found in a simple description of the fact in other words; namely, the sensitivity is lost for a time, the duration of which depends probably among other things, upon the length of the period of avoidance.

One may say that in many instances continual contact with the excitants of nonreaginic food allergy is necessary for the maintenance of that kind of human allergic disease. Vaughan evidently entertains the same view of the phenomenon.

This phenomenon is seldom, if ever, observed in reaginic atopy. It is true that reaginic atopy is sometimes "outgrown" but not necessarily following avoidance. Indeed, it seems well established that the natural process of outgrowing hay fever is often expedited by artificially increasing the exposure to the excitants of it. Under the specific therapy of hay fever by injections of an extract of pollen, the reagin content of the blood diminishes in many patients after a few years of continual (perennial) treatment.⁴

Thus the "latent period" of nonreaginic food allergy stands as a mark of differentiation of this category from the atopic group.

SUMMARY

1. Evidence has been presented of an association of tachycardia with clinical symptoms of familial nonreaginic food allergy, which was so regular in the 31 cases studied that the tachycardia could be used as an objective diagnostic criterion of the allergic reaction through which the specific excitants (foods) could be identified.

2. A "latent period" of lost sensitivity has been recognized in a number of instances of food allergy. In some instances this latent period has been found to vary in duration according to the length of the period of avoidance of the respective allergenic food. The practical use of the latent period is described.

3. Some of the grounds for distinguishing two categories of familial sensitivity are discussed.

4. The occurrence of reaginic and nonreaginic sensitivity to foods in the same individual has been described, and the practical consequences of such a coincidence are discussed.

Addendum (August 25, 1941).—The editors of this JOURNAL have permitted me to withhold this note until the article was about to be printed, in order to extend the period of observation of the patients.

The clinical results can be summarized as follows:

(1) All patients, except M. B., are still under occasional observation.

(2) All patients have had voluntary and blind tests of their specific sensitivity to various (for them) allergenic foods since the completion of their course of treatment by trial diet. In some instances no tachycardia nor allergic symptom followed the test (latent period). In all the other instances there was tachycardia, usually with one or more of the listed allergic symptoms. In most cases the test did not cause *all* the allergic symptoms. Thus, C. T. sometimes suffers tiredness or leg ache without headache or dizziness; sometimes headache alone, sometimes dizziness alone, sometimes both together. These different combinations of symptoms were present on different days under test with a single allergenic food (cane sugar). All symptoms disappeared after that food was eliminated from the diet.

(3) No unexplained allergic symptoms have occurred in any of the patients that have remained under observation.

(4) Some of the patients frequently retest themselves or deliberately "indulge" themselves if the resulting symptoms are not incapacitating; they are able to control their earlier neurasthenia, knowing the cause of each allergic episode.

(5) In some instances one or more of the symptoms that had been exhibited previous to the dietary treatment did not recur at any of the subsequent tests. For example, the monthly petit mal seizures of patient W. C. F. have not recurred once since April, 1940, although beef has been eaten occasionally, causing a moderate tachycardia.

The foregoing summary is, in a sense, an understatement; scientific language does not lend itself to the faithful portrayal of the great improvement described by many of these patients in the state of their "general health," in their sense of "well-being," in their

outlook on life, and in their behavior toward their associates. Of the great importance of these imponderables I am convinced.

Further clinical details of these cases and additional cases, as well as a detailed description of the method of treatment, will be published in a forthcoming monographic report (C. C. Thomas, Springfield, Ill.).

REFERENCES

1. Bulletin of Lederle Laboratories, October, 1939, page 79.
2. Alvarez, Walter C.: Specific Food Sensitiveness, *Am. J. Digest. Dis. & Nutrition* 3: 693, 1936 (Discussion by Arthur F. Cora).
3. Vaughan, W. T.: Food Allergens. III. The Leucopenic Index, Preliminary Report, *J. Allergy* 5: 601, 1934.
- 3a. Vaughan, W. T.: Practice of Allergy, St. Louis, 1939, The C. V. Mosby Co., page 311.
4. Markow, E., and Spain, W. C.: The Effect of Consecutive Years of Treatment Upon Cutaneous Sensitiveness in Late Hay Fever, *J. Allergy* 4: 363, 1933; Further Observations Concerning Effect of Pollen Therapy Upon Cutaneous Sensitiveness in Late Hay Fever, *J. Allergy* 6: 227, 1935.

LEFT VENTRICULAR FAILURE DUE TO ESSENTIAL HYPERTENSION*

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THE most common forms of circulatory failure encountered in the office of the general practitioner are those in which the onset is with failure of the left side of the heart.¹ Failure of the left ventricle results from diseases in which the work of the chamber is increased or its muscle is diseased, and includes such causes as essential and nephritic hypertension, arteriosclerosis, and syphilitic disease involving the orifices of the coronary arteries to the left ventricle, defects of the aortic valve, and mitral disease with predominant regurgitation. Essential hypertension is the most common etiologic factor in the causes of organic heart disease,² and probably accounts for the greatest number of cases of isolated left ventricular failure.

Except as described by Hope,³ the concept of isolated failure of the left ventricle of the heart is one that has arisen within recent years.⁴⁻⁶ White⁶ stated that failure of the left ventricle is more common and important than that of the right ventricle and should be looked for zealously, since by early recognition and proper treatment not only may heart failure of the left side be relieved, but heart failure of the right side, which is likely to follow, may be prevented or postponed.

In a previous study on the types of hypertensive heart failure⁷ in 594 patients it was found that isolated failure of the left ventricle occurred in 156 (26.5 per cent) of the cases. These patients had no signs of systemic congestion. The mortality was lower than among those with combined ventricular failure, and the leading cause of death was not the usual congestive heart failure.

The present clinical study comprised 190 patients with isolated failure of the left ventricle due to uncomplicated essential hypertension. It included 150

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males (79 per cent) and 40 females (21 per cent), whose ages varied from 35 to 77 years at the time of onset of the cardiac symptoms (Table I). Of this group 70.5 per cent were in the 40 to 60 year age group, the so-called "hypertensive age." In a previous study of all types of hypertensive heart disease, 78.8 per cent were in this age group.⁸

TABLE I
PERCENTAGE OF THE AGE GROUPS AT THE ONSET

AGES	MALE	FEMALE	TOTAL	PER CENT
31-40	2	7	9	4.7
41-50	42	13	55	28.9
51-60	67	13	79	41.6
61-70	36	6	42	22.2
71-80	4	1	5	2.6
Totals	150	40	190	100.0

Symptoms which indicated the onset of failure of the left ventricle were dyspnea, cardiac pain (precordial or epigastric or both), weakness, and palpitation. Cardiac pain was a solitary complaint of 20 (10.5 per cent) of the 190 patients, but in all others the cardiac pain, weakness, and palpitation were associated with dyspnea (Table II). White⁶ indicated several important symptoms and signs that point to weakness and failure of the left ventricle, which, he contended, should permit its recognition in the absence of mitral stenosis and congenital heart disease: They are (1) cardiac dyspnea, (2) cardiac asthma or acute pulmonary edema, (3) diminishing vital capacity due to heart disease, (4) engorgement of the roentgen shadows of the lung hilus blood vessels, (5) protodiastolic gallop rhythm at the apex in the absence of heart block, (6) pulsus alternans, and (7) increasing accentuation of the pulmonary second heart sound. Of these seven points the symptoms of dyspnea and cardiac asthma, and the signs of acute pulmonary edema, were the most reliable and helpful in the diagnosis of failure of the left ventricle due to essential hypertension.

TABLE II
FREQUENCY OF THE IMPORTANT SYMPTOMS AND THEIR DURATION

DURATION	DYSPNEA AND CARDIAC PAIN	DYSPNEA	CARDIAC PAIN	DYSPNEA AND WEAKNESS	DYSPNEA AND PALPITATION
1 day to 6 months	50	34	18	7	3
7 months to 1 year	14	6	0	3	3
2 to 5 years	22	14	2	2	3
6 to 10 years	3	6	0	0	0
Totals	89	60	20	12	9
Percentage	46.8%	31.6%	10.5%	6.3%	4.8%

Combined dyspnea and cardiac pain were the most common complaints (46.8 per cent) at the onset of failure of the left ventricle. The mortality was lower among these patients than among those who had the same complaints on the basis of combined failure of both ventricles. The lowest mortality occurred among those who complained solely of dyspnea (31.6 per cent) or of cardiac pain alone (10.5 per cent). Among these patients the mortality was only 13 per cent. Thus the importance of recognizing failure of the left ventricle early,

as either dyspnea or cardiac pain, alone or combined, are the most important symptoms, cannot be overemphasized from the standpoint of attempting to reduce the immediate mortality.

The duration of the failure of the left ventricle, from the onset of the first cardiac symptoms, was estimated in 44 (23.1 per cent) known deceased patients (Table III). Data on 146 (76.9 per cent) living patients were added for comparison. Of the deceased 83.8 per cent died within two years after the onset of cardiac symptoms. The percentage of patients still living, who were observed within two years after the onset of symptoms, was 80.7 per cent, approximately the same ratio.

TABLE III
DURATION OF DISEASE AFTER ONSET OF CARDIAC SYMPTOMS

DURATION	DEAD				LIVING			
	M.	F.	TOTAL	%	M.	F.	TOTAL	%
1 day to 6 months	25	3	28	63.6	61	17	78	53.5
7 months to 1 year	4	1	5	11.4	19	6	25	17.1
2 to 5 years	6	3	9	20.4	28	3	31	21.2
6 to 10 years	2	0	2	4.6	5	7	12	8.2
Totals	37	7	44	100.0	113	33	146	100.0
Percentage	23.1%				76.9%			

Table IV indicates the age at death of these patients; 72.9 per cent died between 40 and 60 years, the "hypertensive age." In the same age period 76.6 per cent of the entire group with hypertensive heart disease⁸ died, which was about the same proportion.

TABLE IV
PERCENTAGE OF THE AGE GROUPS AT DEATH

AGES	MALE	FEMALE	TOTAL	PER CENT
31-40	—	1	1	2.2
41-50	9	1	10	22.9
51-60	19	3	22	50.0
61-70	8	1	9	20.4
71-80	1	1	2	4.5
Totals	37	7	44	100.0

CONGESTIVE HEART FAILURE

In general, congestive heart failure was the cause of death in 65 per cent of deceased patients with hypertensive heart disease.⁸ Among those with isolated failure of the left ventricle the common cause of death was equally divided between coronary thrombosis and congestive failure (Table V). Among 159 cases of hypertensive heart disease with established auricular fibrillation, congestive failure caused 85 per cent of the deaths,⁹ in 36 cases with bundle branch block, it was the cause of death in 97.2 per cent of the deceased;¹⁰ and among 127 patients with hypertensive heart with gross arteriosclerosis, it was the cause of death in 72 per cent of the deceased.¹¹ Since death occurred in only 16 (8.4 per cent) of the 190 cases during the period of observation, congestive heart failure could not be considered a common termination in these patients with isolated failure of the left ventricle. However, it was the cause of death, within six months of the onset, in each of the 16 patients.

TABLE V

PERCENTAGE OF THE CAUSES OF DEATH IN 44 PATIENTS

CAUSE	MALE	FEMALE	TOTAL	PER CENT
1. Coronary occlusion	15	1	16	36.4
2. Congestive heart failure	11	5	16	36.4
3. Uremia	6	0	6	13.6
4. Cerebral hemorrhage	5	1	6	13.6
Totals	37	7	44	100.0

Five illustrative cases are presented to demonstrate that in the absence of any irregular rhythms, complications, or advanced coronary arterial disease, and in spite of treatment, a certain small number of patients with failure of the left ventricle due to essential hypertension died from congestive heart failure.

CASE REPORTS

Patient 1. F. P., a 54-year-old white female, was known to have had hypertension and diabetes for six years. Her blood pressure was systolic 200 and diastolic 100, and the transverse diameter of her heart was 14 cm. at the onset of the first attack of acute failure of the left ventricle fifteen days before her death. In spite of bed rest and adequate doses of digitalis and diuretics, she died of congestive heart failure. Autopsy revealed only a moderately hypertrophied heart, which weighed 490 Gm., hyperemia and edema of the lungs, and cloudy swelling of the myocardium.

Patient 2. H. D., a 54-year-old white male, was known to have had hypertension for five years. At the onset of acute failure of the left ventricle five days before his death the blood pressure was systolic 292 and diastolic 154. There were crepitant râles at both lung bases, and the transverse diameter of his heart was 19 cm. The liver was not palpable and no edema was noted. Following bed rest, and the administration of digitalis and diuretics, no improvement was noted. Five days after the onset he died suddenly. Autopsy revealed a marked hypertrophy of the heart, which weighed 720 Gm., and mild sclerosis of the coronary arteries with occasional fatty and calcified plaques. There was marked edema of the lungs and passive congestion of the liver and spleen. Death was apparently due to congestive heart failure.

Patient 3. A. F., a 56-year-old white male, complained of dyspnea and weakness of three weeks' duration. He had had hypertension for one year. His blood pressure was systolic 210 and diastolic 160. Crepitant râles were present at the bases of both lungs, and the transverse diameter of his heart was 20 cm. After ten days of bed rest, digitalis, and diuretics, he failed to improve. When seen again six weeks later, his blood pressure was systolic 240 and diastolic 160, congestive heart failure was present and persisted until his death within three months. He lived five and one-half months after the onset of isolated failure of the left ventricle. The cause of death was congestive heart failure.

Patient 4. P. D., a 55-year-old white male, known to have had hypertension for four years, complained of dyspnea and precordial pain of five months' span. His blood pressure was systolic 210 and diastolic 160, and the transverse diameter of his heart was 18 cm. The liver was not palpable and no edema was noted. After bed rest and treatment with digitalis he became ambulatory in two weeks. Two months later, in spite of continued digitalis medication, he developed congestive heart failure and died within ten days. Death occurred eight months after the onset of isolated failure of the left ventricle. Congestive heart failure was the cause of death.

Patient 5. R. M., a 58-year-old white male, complained of dyspnea and weakness for eight months. His blood pressure was systolic 250 and diastolic 130, and the transverse diameter of his heart was 19 cm. The liver was not palpable and no edema was noted. His condition did not improve after bed rest, and the administration of digitalis and diuretics. Death occurred eight months and three weeks after the onset of isolated failure of the left ventricle, and was due to congestive heart failure.

CORONARY THROMBOSIS

As a cause of death coronary thrombosis or occlusion was common among the 190 patients with failure of the left ventricle, very common, one might add, as compared with the other groups of patients with hypertension. In general, it occurred as the cause of death in 10 per cent of all deceased hypertensive patients.⁸ Among those with established auricular fibrillation it was noted in only one case (2.8 per cent).⁹ In the cases with bundle branch block it did not occur at all.¹⁰ And among those who had hypertension with gross arteriosclerosis, where coronary occlusion was common, it was the cause of death in only 6.6 per cent of those who died.¹¹

Coronary thrombosis appears to be the important factor in failure of the left ventricle due to essential hypertension. It was the leading additional factor (8.5 per cent) in 190 patients (Table VI). During the course of hypertensive heart disease⁸ it occurred as an additional factor in 6 per cent of the patients; in those with auricular fibrillation⁹ it was noted in only three (1.9 per cent) of the 158 patients; and only one patient (2.7 per cent) with bundle branch block¹⁰ had coronary occlusion. However, in persons with hypertensive heart disease with gross arteriosclerosis, coronary occlusion was more common, since it occurred among 9.5 per cent of 127 patients.¹¹ This was to be expected since the patients in the latter group were older, 84 per cent were over 60 years, and sclerosis of the coronary arteries was the rule rather than the exception.

TABLE VI
ADDITIONAL FACTORS IN 190 PATIENTS

CONDITION	MALE	FEMALE	TOTAL	PER CENT
1. Coronary occlusion	13	3	16	8.5
2. Angina pectoris	10	2	12	6.3
3. Cerebral hemorrhage	9	2	11	5.8
4. Diabetes mellitus	1	4	5	2.6

Cases of isolated failure of the left ventricle with regular rhythm, where death occurred due to the coronary factor, are added to emphasize and illustrate the facts mentioned.

CASE REPORTS

Patient 1. M. W., a 54-year-old white male, had attacks of pain localized strictly to the precordial area for six months. These attacks increased in frequency, and gradually he became short of breath. When he was first examined eleven days before his death, his blood pressure was systolic 190 and diastolic 140. Crepitant râles were heard at the bases of both lungs. The transverse diameter of the heart was 17 cm. The liver was not palpable and no edema was noted. Following bed rest and treatment with digitalis, his condition improved, but on the eleventh day he suffered a typical attack of acute coronary thrombosis. His blood pressure dropped to systolic 146 and diastolic 92, and he died on the same day. An autopsy revealed severe coronary sclerosis, with marked narrowing of the coronary lumen. There was a recent thrombus occluding the left descending branch of the coronary artery at the site of an atheromatous ulcer and a recent myoinfarction in the anterior wall of the left ventricle. The heart weighed 720 Gm. and was markedly hypertrophied. This was a definite case of isolated failure of the left ventricle on the basis of essential hypertension. Death was due to coronary thrombosis while the patient was under treatment and showed marked improvement.

HIGH INCIDENCE OF INFECTIVE STOOLS IN A SMALL OUTBREAK OF INFANTILE PARALYSIS*

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THIS paper is an account of work done on the stools of patients suffering from, or suspected of having, anterior poliomyelitis. The techniques used are based essentially on those developed by Trask, Vignee, and Paul,¹ and by Kramer and his co-workers,² but are modified somewhat to suit our purposes.

Stool specimens were received at the laboratory in 25 ml. sterile glass containers with metal screw tops, or in 250 ml. bacteriologic water bottles with ground-glass stoppers. No reagents were added to the specimens before they arrived at the laboratory. On receipt from 20 to 25 ml. were suspended in from 90 to 100 ml. of sterile distilled water in a wide-mouthed bottle with a ground-glass stopper. To this about 15 ml. of ethyl ether were added. The suspension was then shaken by hand for from five to ten minutes and placed in the refrigerator at a temperature of from 4° to 10° C. After twenty-four hours cultures were made on horse blood agar plates and incubated for twenty-four hours. If these plates showed only slight growth, and no streptococci were present, the suspension was evaporated under vacuum in a desiccator containing sulfuric acid to get rid of the ether. Three mice were inoculated intraperitoneally, each with 0.1 ml. of the suspension. If the mice survived twenty-four hours, it was considered safe to proceed with monkey inoculations. If the mice died, the specimen was treated again with ether, and the whole procedure was repeated. Usually two treatments were sufficient, although before the use of mouse controls two monkeys in the first series died of peritonitis from the lesions of which presumably pathogenic microorganisms were isolated.

Because of these deaths an attempt was made at first to get a better bactericidal agent by using vinyl ether, and also chloroform, but without success; and lastly by shaking the suspension from two to four hours in a shaking machine with ethyl ether. This last procedure sterilized the stools more effectively, but most of them proved to be toxic. The test mice consistently died within ten hours, and only slight bacterial growth was obtained from the peritoneum. Monkeys inoculated with such stools evidenced a severe and rapid toxemia with subnormal temperatures and evidence of paralytic ileus. Two of them died within twenty-four hours, and no microorganisms were isolated either from the peritoneum or from the heart's blood. Moreover, no monkey inoculated with material treated in this manner developed poliomyelitis. We eventually returned to our original method with such success as will be pointed out subsequently.

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The stool inoculations in all cases were made intraperitoneally, from 5 to 20 ml. being given at an injection. Occasionally with two injections on successive days, as much as 30 ml. were given. Stool suspensions shaken only briefly with ether invariably settled out into layers. Upon injection the topmost, clearest layer and some of the middle, cloudier material were taken. The coarse sediment at the bottom of the bottle was not disturbed.

For monkey passage we used rather heavy 10 to 20 per cent suspensions of supposedly infected brain and spinal cord material, ground in the beginning with pulverized glass, but recently with aluminum, and suspended in physiologic salt solution. The suspension was centrifuged at about 2,000 r.p.m. for ten minutes. Routinely 1.0 ml. of the supernatant was inoculated into the left frontal lobe, and 5.0 ml. were inoculated intraperitoneally. In each case, four immature mice were inoculated intracranially and intraperitoneally with the same suspensions. None of the mice at any time showed signs of disease.

The first group of patients consisted of four children with clinical poliomyelitis, and an adult believed to be suffering from a second attack of the disease. All were inmates of an institution and were convalescent. The cases were studied during April, 1939, through the courtesy of Dr. A. C. Silverman of the City Hospital, Syracuse, N. Y. The stool specimens were treated as described, with only slight shaking. Specimens from the four children proved infective for monkeys, and second or third passages were obtained. The animal inoculated with the specimen from the adult remained alive and well. Table I shows the relationships between the clinical disease and the collection of stools. It is of interest that in the case of S. C. a period of ten days elapsed between the probable onset of the disease and the collection of stool material. It is also of interest that all of these children, and another to be noted later, were under two years of age. All had flaccid paralysis of some degree, four in the extremities and one in the face.

The next group of cases studied was from a small outbreak near Germantown, N. Y., in August, 1939, investigated with the help of Dr. L. D. Carpenter. Stool specimens were obtained: one from a child with extensive paralysis in the first week of the disease, and another from a collateral contact who had had a brief febrile illness at about the same time but without paralysis. We were unable to produce poliomyelitis in monkeys, and one of three animals inoculated died of peritonitis; hemolytic streptococci were isolated.

With the cooperation of Dr. H. R. O'Brien and Dr. J. V. Anderson, a third group of cases from Cattaraugus County was studied in December, 1939, and January, 1940. It consisted of seven children in one family, two of them with paralytic anterior poliomyelitis, one recovered from a febrile illness without paralysis, and the other four not clinically affected; and two other individuals, one with residual paralysis six weeks after the onset and another with no paralysis one month following illness. The specimens from this group were obtained from three to six weeks after onset of the disease. They were treated by prolonged shaking with ether, and while no monkeys contracted clinical poliomyelitis, two died of peritonitis and paralytic ileus. No microorganisms could be cultured from either the peritoneum or heart's blood.

During August and September, 1939, a number of stool specimens were received in the laboratory from Buffalo, N. Y., and stored at an average temperature of -5° C. until they could be studied. On January 31, 1940, one of these specimens from an infant ill with paralytic poliomyelitis, received September 9, 1939, was selected and treated by the original method of shaking very briefly with ether. Cultures from the treated suspensions showed no growth. A monkey was inoculated and developed severe anterior poliomyelitis. The virus has been given three subsequent passages. It is of great interest that the virus remained virulent in this stool, frozen in the refrigerator for 124 days, or throughout the winter months.

TABLE I

PATIENT	PROBABLE DATE OF ONSET	STOOL SPECIMEN RECEIVED	MONKEYS INOCULATED FROM STOOL		PASSAGE MONKEYS INOCULATED		PASSAGE MONKEYS INOCULATED	
			ANIMAL NO. AND DATE	ANATOMIC FINDINGS	ANIMAL NO. AND DATE	ANATOMIC FINDINGS	ANIMAL NO. AND DATE	ANATOMIC FINDINGS
<i>Syracuse</i> A. Mc. F. 17 mo.	4/ 9/39	4/30/39	No. 83 5/ 3/39	Anterior poliomye- litis	No. 88 from 83 5/12/39	Meningo- encepha- litis	No. 101 from 83 and 88 12/12/39	Anterior polio- mye- litis
N. W. M. 7 mo.	4/14/39	4/30/39	No. 76 5/ 3/39	Encephalitis	No. 98 from 76 11/21/39	Anterior poliomye- litis		
J. W. M. 14 mo.	4/ 9/39	4/30/39	No. 86 5/ 3/39	Anterior poliomye- litis	No. 91 from 86 5/11/39	Encephalitis. Light an- terior poliomye- litis		
S. C. F. 22 mo.	"About" 4/ 2/39	5/12/39	No. 92 5/12/39	Anterior poliomye- litis	No. 100 from 92 6/ 6/39	Mild en- cephalitis	No. 96 from 92 and 100 12/22/39	Anterior polio- mye- litis
<i>Buffalo</i> E. P. M. 9 mo.	8/23/39	8/31/39	No. 103 1/31/40	Anterior poliomye- litis	No. 113 from 103 3/ 8/40	Anterior poliomye- litis		

Note.—In patients A. Mc. and S. C. the material for final second passage was pooled as indicated, both for fear of losing the strain and to conserve monkeys. It was desired to establish a typical second passage only.

More complete clinical data on four of these cases are discussed by Dr. A. C. Silverman (Am. J. Pub. Health 21: 593, 1941).

Because passage monkeys sometimes fell ill with an acute disease showing involvement of the central nervous system but without flaccid paralysis, no stools were considered to have unequivocally contained the virus of anterior poliomyelitis until at least one typical second or further passage was obtained (see Table I). Thus, even though a monkey showed fever after from five to ten days, accompanied by tremor and other clinical signs of encephalitis, and even though such animals on autopsy showed meningitis with mononuclear cell exudate, perivascular accumulations and nerve cell destruction with neuro-

phagia in the brain stem or cortex but not in spinal cord, the evidence was considered insufficient. Further passages were made until a monkey developed, clinically and histopathologically, typical anterior poliomyelitis. As other authors^{3, 4} have given detailed protocols of the disease in monkeys, it seems superfluous to do so here. Characteristic lesions consisted of neurone necrosis with neuronophagia in the anterior horns and usually elsewhere; perivascular cell accumulations usually of mononuclear cells, but occasionally with polymorphonuclear cells predominating; some degree of leptomeningitis, often of scattered distribution; occasionally small perivascular hemorrhages in the nervous tissue or meninges; signs of microglial activity and elastotodendrosis of astrocytes about local lesions, and various degrees of Nissl's degeneration in ganglion cells. Lesions were observed in all major divisions of the spinal cord, the medulla, pons, mesencephalon, diencephalon, roof nuclei of the cerebellum, and the motor cortex, as well as in dorsal root ganglia and Gasserian ganglia. No lesions were seen in the thoracolumbar autonomic ganglia.

In many monkeys focal interstitial nephritis was observed. Similar lesions, however, have since been found in a monkey dying of unknown cause, and in two monkeys dying of bacillary dysentery.

All intracranial inoculations in monkeys (*Macacus rhesus*) were made into the left frontal lobe. All monkeys were sacrificed by light ether anesthesia and intracardiac air embolism. None were permitted to die spontaneously. Autopsy in each case was performed as soon as death supervened. In addition to the parts of the nervous system mentioned sections were taken routinely from all visceral organs, including the major divisions of the gastrointestinal tract, and from the peritoneal lymphatics. The viscera were fixed in Zenker-acetic solution and stained with hematoxylin and phloxine. Central nervous system tissue was fixed in Zenker-acetic solution, formalin, ammonium bromide, and 70 per cent alcohol. Hematoxylin-phloxine and thionine stains and silver carbonate and gold-chloride sublimate impregnations were done in each case, and when it seemed indicated, scarlet red stain (for demonstration of fat) was made. In only one instance, however, was any considerable fatty change observed.

All autopsies were controlled by cultures and film preparations from the peritoneum, brain, and heart's blood on horse blood agar. In no case were any significant bacterial findings observed. All passage suspensions were controlled by culturing on horse blood agar, with no significant bacterial findings. All inoculations into monkeys were controlled by parallel inoculations into young Swiss mice. The mice were observed for one month. No instances of disease were noted. Virus strains, if one may use the term in this connection, from three different cases were put intracranially and intraperitoneally into Eastern cotton rats (*Signodon hispidus hispidus*), but no successful passage was obtained.

Serial "faith" passages through six passage generations of cotton rats yielded no results. Cotton rats and young Swiss mice intoxicated with guanidine hydrochloride, diorthocresylphosphate, cadaverine hydrochloride, and ethylenediamine dihydrochloride, all failed to contract the disease. All were observed for a month following intracranial and intraperitoneal inoculation with tissue

containing virus. All chemical intoxications were maintained over a period of from ten days to two weeks, starting usually five days previous to exhibition of the virus. Three rats put on thyroxine injections all died during manipulation for taking temperatures before poliomyelitis would have been expected to develop.

It is worth noting here that many of our cotton rats died suddenly during or after very brief handling. We were unable to find a cause for death on autopsy, though it may be significant that immediately post mortem there was marked peristaltic activity observable through the intact abdominal wall.

SUMMARY AND CONCLUSIONS

Single random specimens of stool were obtained from a total of 17 cases of typical, abortive, or atypical suspected anterior poliomyelitis and inoculated into monkeys, with five "takes." Nine of these cases had clinical paralysis. Two were clinically abortive types. Two were atypical. Four were contacts without clinical signs or symptoms.

Eight stool specimens were treated by the method outlined, with shaking from five to ten minutes with ether. Of these eight cases, seven had paralysis. From five of the paralyzed cases, successful transfers of the disease into monkeys were obtained.

Nine stool specimens were treated by the method outlined, but the suspensions were shaken for two to four hours with ether. Of these nine cases, two were paralytic; none of the inoculated monkeys showed signs of poliomyelitis.

One of the infective stool specimens was collected as late as forty days after the probable onset of the disease.

One of the stool specimens kept at a temperature of -5° C. remained infective for 124 days after collection.

All stool and monkey cord passages were controlled by mouse inoculation.

All successful passages were from the stools of infants under two years of age, in four cases at least, on similar diets.

It does not appear to me that any deductive conclusions may be drawn with certainty at this stage of the investigations. However, the question under what conditions poliomyelitis virus will remain infective in stools seems to be of interest.

REFERENCES

1. Trask, J. D., Vignee, A. J., and Paul, J. R.: *Poliomyelitis Virus in Human Stools*, J. A. M. A. 111: 6, 1938.
2. Kramer, S. D., Hoskwith, B., and Grossman, L. H.: *Detection of the Virus of Poliomyelitis in the Nose and Throat and Gastro-intestinal Tract of Human Beings and Monkeys*, J. Exper. Med. 69: 49, 1939.
3. Flexner, Simon, and Lewis, P. A.: *Experimental Epidemic Poliomyelitis in Monkeys*, J. Exper. Med. 12: 227, 1910.
4. Hurst, E. W.: *The Histology of Experimental Poliomyelitis*, J. Path. & Bact. 32: 457, 1929.

BEHAVIOR OF THE RECIPIENT'S LEUCOCYTE COUNT FOLLOWING TRANSFUSION OF PRESERVED BLOOD*

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THE increasing use of preserved blood for transfusions has raised many questions regarding its effectiveness as compared to that of fresh blood. Among these questions is its relative value in agranulocytosis and in septicemia or other infectious conditions, both as regards the transferal of immune bodies and the leucocytes themselves. Although there is still a difference of opinion as to the effectiveness of any transfusion in combating an infection, the procedure is frequently used.

It has been shown that with a transfusion of fresh blood the transfused leucocytes disappear from the recipient's blood stream within two and one-half hours and that such a transfusion does not cause a significant increase in the leucocyte count.¹ Since the life of a normal leucocyte is from one to five days, and the number of these cells in the ordinary transfusion is relatively small, one could not expect a significant increase in the recipient's count. With preserved blood it has been shown that there is a gradual disintegration and reduction in the number of leucocytes during the period of storage, and it has been recommended that this blood not be used in patients with agranulocytosis.² The value of transfusions of preserved blood in infectious states has also been questioned, since the degenerative changes in the neutrophils reduce their phagocytic activities.³ On the other hand, it has been suggested that the products of disintegration may stimulate the formation of new cells in the recipient.²

In order to determine the effect of transfusion of preserved blood on the recipient's circulating leucocytes, counts were made before and after the giving of blood from the "blood bank." The age of the blood varied from one to twenty-five days, as is noted in the tables, and the preservatives used were either sodium citrate or a dextrose-citrate mixture, as described by DeGowin.⁴ The patients selected for study were those without an acute infection or recent hemorrhage. No patient was studied during a postoperative period. In one group of 15 patients leucocyte counts were done at two-hour intervals following transfusion, and in a second group of 12 patients counts were done at twenty-four-hour intervals, so that information might be obtained on either an immediate or a delayed rise due to stimulation from the disintegration products. The results are shown in Tables I and II.

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TABLE I
LEUCOCYTE COUNTS AT TWO-HOUR INTERVALS

CASE	INITIAL COUNT		2 HOURS	4 HOURS	6 HOURS	8 HOURS	AGE OF BLOOD DAYS	DIAGNOSIS
1	7,300	Transfusion	7,050	10,050	8,200	7,050	22	Carcinoma of cervix
2	1,700		1,500	1,850	1,250	2,000	21	Aplastic anemia
3	2,400		1,950	1,650	2,450	1,900	1	Aplastic anemia
4	7,300		8,050	7,800	8,500	8,100	7	Peptic ulcer
5	4,000		4,300	5,100	5,600	4,550	6	Peptic ulcer
6	5,450		4,950	4,450	5,700	4,800	5	Peptic ulcer
7	5,250		5,650	6,700	5,050		25	Carcinoma of colon
8	8,300		8,500	7,100	7,500		25	Incomplete abortion
9	5,600		5,750	4,410	4,000		2	Leucemic leucemia
10	10,650		10,750	11,200	10,800		3	Carcinoma of stomach
11	3,350		3,950	3,400	3,100		4	Aplastic anemia
12	12,950		13,680	13,900	11,950		4	Chronic cholecystitis
13	2,000		1,650	2,100			23	Aplastic anemia
14	11,300		11,500	10,800			19	Ulcerative colitis
15	10,050		10,650	10,800			2	Carcinoma of stomach

TABLE II
LEUCOCYTE COUNTS AT TWENTY-FOUR-HOUR INTERVALS

CASE	INITIAL COUNT		24 HOURS	48 HOURS	72 HOURS	96 HOURS	120 HOURS	AGE OF BLOOD DAYS	DIAGNOSIS
1	8,500	Transfusion	9,600	9,400	10,100	11,200	9,000	1	Pernicious anemia
2	6,900		7,200	8,200	6,350	6,900	7,500	6	Peptic ulcer
3	7,200		7,000	7,800	6,350	7,750	6,250	6	Carcinoma of cervix
4	6,300		7,050	8,100	7,400	7,000	7,650	22	Carcinoma of cervix
5	10,200		10,550	9,350	8,050	8,400		3	Nutritional anemia
6	12,350		8,900	9,450	8,800	8,200		16	Carcinoma of stomach
7	5,250		5,850	4,250	3,600	3,700		25	Carcinoma of colon
8	3,600		3,800	3,400	3,900	3,700		2	Leucemic leucemia
9	3,350		3,750	3,300	2,950	3,650		4	Aplastic anemia
10	11,300		9,650	10,050	10,350	10,050		19	Ulcerative colitis
11	8,450		9,450	8,660	7,900			10	Hypochromic anemia
12	8,300		7,600	7,000	7,900			25	Incomplete abortion

Examination of these tables reveals that there was no consistent change in the leucocyte count as a result of the transfusion. An elevation in the count occurred in some cases, but this was not constant; in other cases a reduction in the count was noted. These variations are no greater than can be accounted for by the unavoidable error in the method, the diurnal variations, or the rhythmic variations in the circulating leucocytes.⁵⁻⁷ The results are in accord with what one would expect from our knowledge of the life of the leucocytes and their behavior during storage. The age of the blood made no difference in the results nor did the type of case to which it was given. In aplastic anemia one could expect no latent rise from the stimulation of disintegration products, and although such a reaction might occur in cases with normal bone marrow, it was not encountered in this group of cases.

Transfusions of preserved human blood produce no constant or significant changes in the leucocyte count of the recipient.

REFERENCES

1. Castle, W. B., and Minot, G. R.: *Pathological Physiology and Clinical Description of the Anemias*, Oxford Medicine 2: 680 (39).

2. Belk, W. P., Henry, N. W., and Roenstein, F.: Observations on Human Blood Stored at 4 to 6 Degrees Centigrade, *Am. J. M. Sc.* 198: 631, 1939.
3. Kolmer, J. A.: Preserved Citrated Blood "Banks" in Relation to Transfusion in the Treatment of Disease With Special Reference to the Immunologic Aspects, *Am. J. Med. Sc.* 197: 442, 1939.
4. DeGowin, E. L., Harris, J. E., and Plass, E. D.: Studies on Preserved Human Blood. I. Various Factors Influencing Hemolysis, *J. A. M. A.* 114: 850, 1940.
5. Medlar, E. M.: The Extent of the Variations in the Leukocytes of Normal Individuals, *Am. J. M. Sc.* 177: 72, 1929.
6. Shaw, A. F. B.: The Diurnal Tides of the Leukocytes of Man, *J. Path. & Bact.* 30: 1, 1927.
7. Sabin, F. R., Cunningham, R. S., Doan, C. A., and Kindwall, J. A.: The Normal Rhythm of the White Blood Cells, *Bull. Johns Hopkins Hosp.* 37: 14, 1925.

THE EFFECTS OF DECREASED TEMPERATURE ON THE ACTIVITY OF INTACT MUSCLE*

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SINCE muscular contraction involves intrinsic chemical and physical changes, the influence which a fall in temperature has on muscular activity may be anticipated by studying the effects which temperature changes have on the processes involved in muscular contraction. However, a mere statement of the qualitative effects which temperature changes have on muscular contraction is inadequate for a complete explanation of poor performance, and also muscle injury, experienced by individuals who are called on to exercise strenuously.

In order to have a clearer picture of the effects of lowering the temperature of a muscle on its activity, the experiment herein reported was performed. To make the experimental data parallel the conditions as they actually exist, the activity of the intact gastrocnemius muscle and its antagonist when exposed to cold, was studied. In order to determine the effects of lowering the temperature of the muscle on its activity, the form curve of a normal muscle was compared with that of the same muscle after it has been cooled for various periods of time.

The gastrocnemius muscle was caused to contract by applying submaximal break induction shocks to it. This was accomplished by attaching one pole of the inductorium to an electrode placed over the medial motor point of the muscle, and the other pole to an electrode placed on the thigh, over an indifferent area. A foot switch was employed in the primary circuit so that the experimenter's hands were free to manipulate the recording apparatus.

The subjects were placed in a prone position on a table with the feet well over the edge. A cord was attached to the toe of the subject's shoe, thence over a pulley under the table to a recording stylus suspended from a rubber band. The rubber band furnished the means by which uniform tension was maintained on the muscle throughout the experiment. A signal magnet, placed in the primary circuit, was superposed above the recording stylus. A 100 double vibration electrically driven tuning fork was placed between the recording stylus and the signal magnet. The record was made on an extension kymograph which

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was operated manually. Obviously, the records were recorded upside down, since a contraction of the muscle caused an extension of the foot, thus pulling the recording stylus down.

The procedure in securing the data was first, to make control records from the muscle, and then pack the leg in ice. After five, ten, and twenty minutes of cooling, additional records were made. Fifteen to twenty tracings were secured under each condition studied.

By means of ordinates the response of the muscle was divided into its latent period, periods of contraction and relaxation. The length of each period was determined by counting the number of tuning fork vibrations between the ordinates.

THE DATA

Data were collected from 12 individuals. The values given in Table I are the means of no less than 15 responses per period for each individual.

TABLE I

A COMPARISON OF THE EFFECT OF COOLING ON THE PERIODS OF RESPONSE OF THE INTACT GASTROCNEMIUS MUSCLE

	LATENT TIME	PER CENT INCREASE	CONTRACTION TIME	PER CENT INCREASE	RELAXATION TIME	PER CENT INCREASE
Normal	0.024 ± 0.002		0.089 ± 0.006		0.267 ± 0.034	
Cooling 5 min.	0.027 ± 0.002	12.5	0.108 ± 0.012	21	0.432 ± 0.035	62
Cooling 10 min.	0.030 ± 0.002	25	0.130 ± 0.015	46	0.574 ± 0.045	115
Cooling 20 min.	0.033 ± 0.002	37.5	0.162 ± 0.024	82	0.726 ± 0.061	172

Although the well-known facts relative to the effects of cooling muscle are demonstrated by the experiment, the phase of activity in which we are most concerned in this investigation is the period of relaxation. After five minutes of cooling the relaxation time is increased 62 per cent, while the period of contraction and the latent time are increased 21 per cent and 12.5 per cent, respectively. This means that relaxation is affected three times as much as contraction and five times as much as the latency of the muscle. Ten minutes of cooling increases the period of relaxation 115 per cent, while the contraction time is increased only 46 per cent and the latency 24 per cent. Under this condition, relaxation is increased 2.5 times as much as contraction and 4.6 times that of the latency. After twenty minutes of cooling the situation is similar. Relaxation is increased 172 per cent, contraction 82 per cent, and latency 37.5 per cent. Under this condition, relaxation is increased 2.1 times as much as contraction and 4.6 times as much as latency. In view of these facts one is justified in emphasizing that cooling a muscle not only affects the relaxation more than any other period of its activity, but also slows this period from two to three times as much as the contraction.

The effect of cooling on muscular relaxation is especially important to athletes who participate in sports, such as sprinting, during which the muscles perform explosive movements. As far as the latent and contraction periods are concerned, the warming-up of a muscle may be adequate, but from the point of view of relaxation at that stage it is totally unprepared for explosive action. Such a condition makes for poor performance, since a succeeding contraction cannot occur until the muscle relaxes from the preceding contraction.

A complete warm-up also is important from the standpoint of muscle injury, since when the driving muscles contract, their antagonists must relax in a synergistic fashion. Injury to an improperly warmed-up antagonist is not uncommon. Such injuries can only be prevented by sufficient preliminary exercises to reduce the relaxation time of an antagonist commensurate with the reduction in contraction time.

It is also worthy of note that muscle injury may occur if a properly warmed-up muscle is exposed to a cold environment. Such occurrences as these can be prevented only by protection from cold. In fact, many sprinters, running from a protected environment into a cold draft or breeze, have been observed to have experienced muscle injury.

SUMMARY AND CONCLUSIONS

An investigation of the effect of cooling intact muscle shows that the lowering of the temperature increases the relaxation time two to three times as much as the contraction time of the muscle. Inadequate relaxation due to an improper warm-up is sufficient to explain poor performance in explosive bouts of exercise such as sprinting. Also, injury to muscles antagonistic to driving muscles is explained on the basis of a prolonged relaxation time, due to either an inadequate warm-up, a cold environment, or a sudden change in the environment.

A STUDY OF THE THERAPEUTIC EFFECT OF SULFAPYRIDINE IN PNEUMOCOCCUS INFECTED MICE IN ATMOSPHERES OF VARYING OXYGEN TENSION*

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INTRODUCTION

INVESTIGATIONS on the mechanism of the action of sulfanilamide and its derivatives *in vitro* have led to the theory that an oxidation product of the drug is responsible for the bacteriostasis noted (Mayer;¹ Ottenberg and Fox;² Fox, Cline, and Ottenberg³). As a corollary to this theory the bacteriostatic action of the drug should be lessened under anaerobic conditions of culture. Swift, Lancefield, and Goodner⁴ found that with 6 strains of hemolytic streptococci, if removal of oxygen and reduction are complete before inoculation of the media with the organisms, bacteriostasis by sulfanilamide is prevented. Shinn, Main, and Mellon⁵ demonstrated that reduction of the oxygen in superambient air of broth cultures of type I pneumococcus reduced or prevented bacteriostasis by sulfanilamide. This inhibition of bacteriostasis was also noted under complete anaerobic conditions. When, however, the superambient air contained 0.04 per cent oxygen, this inhibition effect was absent. Fox,⁶ studying

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this same phenomenon, concludes that complete initial deprivation of oxygen interferes with bacteriostasis and increased oxygen availability magnifies bacteriostasis. Unpublished studies by Fox⁷ on the effect of oxygen deprivation on the action of sulfapyridine on pneumococci demonstrate that the action of the drug is lessened under anaerobiosis. This is in disagreement with Long,⁸ who could not demonstrate this effect. Long's report shows that in both aerobic and anaerobic cultures he obtained sterility. Using streptococcus as the test organism, Long finds a lessened bacteriostatic action of the drug under anaerobic conditions.

On the basis of this work it was felt that perhaps a reduction of the oxygen saturation in the blood of mice would similarly interfere with the action of sulfapyridine on pneumococcus septicemia. Conversely, an increase in oxygen saturation should enhance the bacteriostasis.

EXPERIMENT 1

Infecting dose: 100 M.L.D.

OXYGEN PER CENT	NO. OF MICE	TREATMENT	DEATH IN DAYS										NO. DIED	NO. SUR- VIVED
			1	2	3	4	5	6	7	8	9			
20.8	9	Infected (control)	9	0	0	0	0	0	0	0	0	9	0	
20.8	15	Infected and drug	1	0	1	0	1	4	3	2	2	14	1	
8	15	Infected and drug	3	2	1	1	1	2	2	1	0	13	2	
60	13	Infected and drug	1	1	1	2	0	3	1	3	0	12	1	

EXPERIMENT 2

Infecting dose: 1,000 M.L.D.

OXYGEN PER CENT	NO. OF MICE	TREATMENT	DEATH IN DAYS									NO. DIED	NO. SUR- VIVED
			1	2	3	4	5	6	7	8	9		
20.8	17	Infected (control)	17	0	0	0	0	0	0	0	0	17	0
20.8	17	Infected and drug	0	1	0	3	4	4	2	2	1	17	0
8	17	Infected and drug	4	1	2	1	4	3	2			17	0
60	17	Infected and drug	1	1	2	3	5	1	2	1		17	0

METHODS

For these experiments we used a mucoid type I pneumococci which was passed through rabbits once a month and kept in rabbit's blood under vaseline seal. For infection a subculture was made using 0.5 c.c. of this stock culture. The infecting volume was 0.3 c.c. of the given dilution (in broth) of a five-hour subculture, injected intraperitoneally.

The drug sulfapyridine was suspended in 10 per cent gum acacia and fed twice daily, giving 30 mg. of the drug per day for six days and 15 mg. daily for the seventh and eighth days. The given atmospheres were continued for twenty-four hours after the last drug dose, and the experiment was considered complete twenty-four hours after discontinuation of the gases. In all cases, cultures from heart blood were taken at autopsy and the organisms were recovered. In each of the experiments a group of normal mice was put in with the low oxygen group to control deaths due possibly to the low oxygen.

To control the factor of the effect of varying the oxygen atmosphere on pneumococcus septicemia, three groups of 15 mice each were infected with 100 M.L.D. of the culture and subjected to 8 per cent oxygen, 60 per cent oxygen, and room air (20.8 per cent), respectively. In all groups the mice died between fourteen and twenty-four hours. The distribution of deaths was approximately the same in all three groups.

Varying the oxygen concentration of the atmosphere did not appear to affect the mortality rate.

Five additional similar experiments were run in which a total of 225 mice were exposed to low and high oxygen percentages, and the conditions in Experiments 1 and 2 duplicated. Since the results in these experiments were confirmatory of the foregoing data, a detailed presentation is not given.

CONCLUSIONS

In cultures of pneumococci anaerobic conditions have been reported to prevent the bacteriostatic action of sulfapyridine.

Pneumococcus-infected mice were treated with sulfapyridine in atmospheres of low oxygen tension (8 per cent), air and high oxygen tension (60 per cent) without significant effect on the mortality rate. Varying the oxygen tension of the atmosphere does not appear to modify the effectiveness of sulfapyridine in pneumococcus septicemia.

It is acknowledged that these results do not apply to pneumonia in which the oxygen tension of the capillary blood may fall to far lower levels.

REFERENCES

1. Mayer, R. L.: *Biol. med. (Suppl.)* 27: 45, 1937.
2. Ottenberg, R., and Fox, C. L.: *Proc. Soc. Exper. Biol. & Med.* 38: 479, 1938.
3. Fox, C. L., Chne, J. E., and Ottenberg, R.: *J. Pharmacol. Exper. Therap.* 66: 99, 1939.
4. Swift, H. F., Lancefield, R. C., and Goodner, K.: *Tr. A. Am. Physicians* 1: 217, 1935.
5. Shinn, L. E., Main, E. R., and Mellon, R. R.: *Proc. Soc. Exper. Biol. & Med.* 40: 640, 1939.
6. Fox, C. L.: *Am. J. M. Sc.* 199: 487, 1940.
7. Fox, C. L.: Personal communication.
8. Long, P. H.: *Chemistry and Medicine*, edited by M. B. Visscher, Univ. Minnesota Press, 1940, page 195.

GOLD COLLOID AND COLLOIDS OF OTHER HEAVY METALS IN THE TREATMENT OF RHEUMATOID ARTHRITIS*

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THE rapidly accumulating literature on the use of gold salts in rheumatoid arthritis is in apparent harmony on the following two points: (1) Gold in the form of its salts is perhaps the most efficient agent known in the treatment of rheumatoid arthritis. (2) The toxic or untoward reactions so frequently encountered are a serious drawback to the use of this drug. The clinical results¹ obtained with gold sodium thiomalate in a recent investigation undertaken with the purpose in view of reducing the frequency and severity of toxic phenomena served to strengthen these views.

With the two enumerated points in mind, therefore, I was induced to search for some similar or related agent, or agents, that might incorporate all the beneficial therapeutic qualities of gold salts without their serious toxic effects. It was also my aim to ascertain whether the beneficial attributes of gold were due to something inherent in the gold radical alone, regardless of chemical affinity, or whether these attributes were likewise demonstrable in the radicals of other heavy metals such as platinum, bismuth, etc.

Therapeutic research on the use of gold for some unascertained reason appears to have been limited to the salt. Review of the literature failed to discover any published reports on the treatment of rheumatoid arthritis with the colloid of gold or of other heavy metals. Several authors have reported using "colloidal" gold, but on closer investigation it was found that the term "colloid" had been used somewhat indiscriminately.

As is well known, the inorganic salts of gold are very reactive chemically. Most of them decompose readily, with the liberation of metallic gold, while even those which are generally regarded as fairly stable are quite reactive. As an example, a dilute solution of gold chloride, AuCl_3 , decomposes on heating, while on exposure to direct sunlight metallic gold is precipitated and hydrogen peroxide is formed in the solution. The chemical reactivity of the somewhat more stable aurichloric acid, HAuCl_4 , was observed by Boyle in 1663, who found that it would color the skin, nails, or ivory a purplish-red tint when exposed to light. This, of course, immediately calls to mind the staining of the skin experienced with solutions of silver nitrate. Even gold sodium thiosulfate, which has achieved a definite measure of recognition in the treatment of lupus erythematosus, is unstable, its solutions becoming yellow and decomposing on standing.

*From the Arthritis Clinic, St. Mary's Hospital, Brooklyn, William McCollom, M.D., attending physician.
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The colloid solutions for this study were especially prepared by Crookes Laboratories, Inc., New York, N. Y.

It seems obvious, therefore, that in gold we have a highly reactive element, and that this reactivity may in part be responsible for the considerable toxicity of its salts. The thiomalate with which my associates and I have had considerable experience is an organic salt of gold which does not seem to be quite so reactive as the thiosulfate, and which in consequence can be, and is, used in considerably larger doses. However, even the thiomalate must be administered with considerable caution.

In view of this toxicity it seemed desirable to explore the possibility of introducing gold in a form which was less reactive chemically and which, in consequence, might permit a more sustained, effective, and less toxic form of therapy. Colloidal gold appeared to offer definite possibilities.

Within recent times reports on the use of dilute solutions of colloidal gold in the treatment of malignancy have been published,²⁻⁸ and although the results obtained have in no way been conclusive, there are indications that even in the dilute solutions employed colloidal gold exerts a definite analgesic action.

Although metallic gold itself is a very stable metal, being unaffected by air and water and many ordinary laboratory reagents and solvents, it is not absolutely insoluble. When exhibited as a colloid, this element consists of innumerable particles of metallic gold. It seemed, therefore, particularly in this extremely subdivided form, that when injected in the body it would act in a manner similar to that ascribed to colloidal silver.

It will be remembered that when colloidal silver was made available commercially, new therapeutic fields were created for this element, which, in the form of its soluble salts, was far too reactive and toxic for use in more than very dilute solutions, except where a definitely caustic action was desired.

New and Nonofficial Remedies, referring to colloidal silver preparations, points out that the silver in these colloidal suspensions does not exist to any great extent in the free ionic state, and, therefore, does not precipitate chlorides and proteins. As a result it is noncorrosive and nonirritant, yet retains a definite degree of antiseptic action. This silver action is not proportionate to the total silver content of the colloidal silver preparation and varies with the different colloidal silver compounds. The antiseptic action of these colloids is undoubtedly due to the liberation of continuous, very low concentrations of silver ions.

Weiser⁸ states that the more finely subdivided the silver, the stronger its bactericidal action. By analogy, therefore, colloidal gold, because it is almost devoid of gold ions and consequently reduced of its chemical reactivity, should not react immediately with the albumen, proteins, and salts which are found in the body, thereby permitting intramuscular administration without any immediate local irritation or general reaction.

My experience indicates this to be true. Furthermore, the billions of tiny particles of metallic gold should conceivably act as depots for the formation of gold ions, to which part of the action of gold salts may be ascribed, resulting in a more sustained action of the colloid but of a somewhat lower intensity than that obtained with gold salts.

There is another factor which might, theoretically, impart to a colloid of gold a beneficial action probably not associated with gold salts. It is well

known, for instance, that the injection of any colloid into the blood stream is attended by a certain amount of shock similar to that which usually follows the injection of a nonspecific protein. Thus, when using colloidal gold, with the therapeutic action of the gold itself, one is able to combine a mild nonspecific reaction.

Colloidal platinum (at.wt. 195.2) and colloidal bismuth (at.wt. 209.0) were included with gold in this study, since these metals have atomic weights approximating that of gold (197.2), and since it was thought possible that some common heavy metal action might be demonstrable therapeutically. Selenium, though not a heavy metal, because of its vaunted analgesic action in malignancies, which seemed to correspond in some way with this action of colloidal gold, was also included in this study. Selenium, however, together with platinum and bismuth, gave insufficient promise of therapeutic usefulness and was discontinued early in the study. Lead and some of the other metals in the higher scale of atomic weights were discarded as probably being too toxic.

It was felt that at least a period of two years from the initiation of this work should elapse before a more or less accurate evaluation of the therapeutic efficacy of the agents employed could be attempted with any degree of accuracy.

The duration of treatment in each individual case varied from three to twelve months. As with the salt, treatment consisted of three series of injections. Each series comprised 1 Gm. of metallic gold, an interval of six weeks being interposed between each series. An average of approximately 1 Gm. of gold was administered in each individual case.

In the beginning of this work a 0.1 per cent solution of colloidal gold was injected in 5 c.c. doses intragluteally. With this relatively dilute solution the actual amount of gold administered was very small, amounting to no more than 5 mg. (0.005). In spite of the relatively large quantity of solution injected, however, it was felt that larger doses of metallic gold, equal to those used by other investigators and myself, should be employed if an actual comparison of therapeutic results was to be obtained. Therefore, a second and more concentrated 1 per cent solution was prepared in which 2.5 c.c. of the suspension corresponded to 0.025 Gm. of metallic gold. This more concentrated solution in a number of cases resulted in an unsightly coppery discoloration at the point of injection, and for this reason had to be administered intragluteally. In both these suspensions the gold was "stabilized" with an emulsoid colloid containing 0.3 per cent trieresol.

For the sake of this therapeutic comparison it was deemed unfortunate that larger amounts of gold corresponding to the higher dosage brackets (0.05 to 0.1 Gm.) employed by Forestier and other investigators were not used at this time. In justification it must be remembered that in this study of the therapeutic effects of colloidal gold in rheumatoid arthritis we were treading, more or less, on virgin soil, and for that reason, had to exert a greater amount of caution. In passing, it must be mentioned, however, that far larger doses than those here employed have been used in the treatment of carcinoma and other malignancies.

International Atomic Weights

Au	Pt	Bi	Ir	Pb	Hg	Ra	Th	(Sr)
197.2	195.2	209.0	193.1	207.2	200.6	226.0	232.15	(79.2)

Metals Administered

Total No. Patients Treated	Au	Pt	Bi	Se
50	36	4	4	6

Results of Colloidal Gold Therapy in Rheumatoid Arthritis

Total No. Cases Treated	Unimproved	Slight	Moderate	Marked	Uncertain
36	9	4	2	19	2

Fifty patients were injected with the above-mentioned colloids. Of these, 36* received gold, 4 platinum, 4 bismuth, and 6 selenium. Practically all these patients had received some previous form of therapy with unsatisfactory results. Two had been treated with gold salts in the form of gold sodium thiomalate intramuscularly, and two had had gold sodium thiosulfate with equally disappointing results. Among the series treated with the gold colloid were included two cases of generalized fibrositis that for years had failed to respond to any other form of therapy. The duration of the disease varied from three months to fifteen years. As to the severity or the grade of involvement of the rheumatic process, it might be said that the patients conformed to the average run of chronic cases currently seen in a large metropolitan clinic. The pathology varied from marked deformities as found in patients bedridden for a number of years, to those milder forms merely showing periarticular soft tissue changes. The usual laboratory procedures essential to the use of gold salts, i.e., sedimentation rate, complete blood studies, and urinalysis, were carried out before, during, and after initiation of therapy. Of 36 patients treated with gold colloid, 19, or 52.7 per cent, showed a definite improvement, while 9, or 25 per cent, failed to show any degree of amelioration. The two patients suffering from fibrositis demonstrated a quick and gratifying response.

Statistics in over 1,600 persons treated with gold salts show that there is an average marked improvement of about 54 per cent and a total lack of improvement in approximately 13 per cent. Considering the relatively small number of cases presented in this preliminary report, the difficulty of drawing valid conclusions as to comparative therapeutic value of gold salts and the colloid of gold is apparent, and any inferences derived from this study must have, therefore, only a relative value.

DISCUSSION

Of the metallic colloids employed, gold, because of its apparent therapeutic superiority over the other afore-mentioned metals, received predominant attention.

In drawing a differential comparison between gold salts and the colloid of gold, the following three criteria appear of outstanding importance: (1) therapeutic efficiency, (2) toxicity, (3) biological action. What the results are with the use of gold salts is common knowledge and repetition appears unnecessary.

*Seven of these patients were treated in the arthritis clinics of the Brooklyn and Post Graduate Hospitals, Doctors G. E. Anderson and Edward Hartung, respectively, in charge.

(1) *Therapeutic Efficiency of Colloidal Gold*.—In keeping with a definite subjective improvement in the majority of persons treated, there was a tendency toward regression of those signs which together comprise the rheumatoid picture. As in the case of gold salts, this regression consisted of reduced pain and peri-articular swelling as well as of increased function of the parts involved. It was my impression, however, that the therapeutic response was lacking the quick, dramatic character often observed with gold salts.

(2) *Toxicity*.—In contrast to the highly toxic nature of gold salts, as occasioned by their ready solubility and intimate chemical action on the body tissues, thereby detracting from their value as routine therapeutic agents, the colloid, in the individual and total doses already mentioned, gave little evidence of such toxicity. This almost complete lack of toxicity is in conformity with the physico-chemical structure of all colloids, which, as has already been mentioned, are, chemically, relatively inert substances and therefore exert little, if any, destructive action on the body tissues. With the colloid, for instance, there were none of those toxic, and sometimes alarming, clinical manifestations so frequently obtained with gold salts. In the entire series treated, one patient developed an enlargement of the axillary lymph glands. This patient, upon investigation, had been previously treated with gold salts. This glandular reaction has been found to be not an uncommon sequel to the use of the salt as well.

(3) *Biological Action*.—Biological action as determined by the sedimentation rate, complete blood studies, and urinalysis are as follows: As with gold salts, there was a parallelism between the drop in sedimentation rate and clinical improvement. This drop, however, in keeping with the slower therapeutic response, was definitely delayed.

At no time did any marked or abnormal changes occur in the urinary picture. If one realizes that a good part of any heavy metal, such as gold or mercury, is eliminated through the kidneys, one is again struck with the basically inert nature of the metallic colloids.

The essentially different nature of gold salts as compared with colloidal gold has been further emphasized by bacteriostatic tests with the sera of patients treated with both these substances. For instance, the sera of patients treated with gold salts showed a very active bacteriostatic power even after as few as three injections. In contrast, the colloid-treated serum utterly failed to retard the growth of organisms. These experiments conducted independently by Hartung and Sabin were in complete accord on the results obtained. This, in my opinion, is not necessarily proof of the inefficacy of the colloid. The very nature of its action, i.e., slower ionization, greatly diminished chemical reactivity, etc., would eliminate any attributes of great bacteriostatic power, especially after merely three or four injections.

MECHANISM OF COLLOID THERAPY IN RHEUMATOID ARTHRITIS

Studies of the reticulo-endothelial system in the deposition of colloidal and particulate matter in articular cavities furnish us with some very interesting data. Kuhns and Weatherford¹⁰ describe experiments dealing with the deposition of trypan blue in the reticulo-endothelial system in and about the joints of the albino rats. These authors concluded that the colloidal and particulate matter used in their experiments is carried by the blood stream from various

parts of the body and stored in the histiocytes of the synovial membrane. Some amounts are also stored in the bone marrow, intermuscular septi and articular fat pads. Inflammation of the articular tissues tends to increase the amount of particulate matter deposited. The inferences which follow these experiments are that stimulation of the reticulo-endothelial system might be effected by the deposition of colloidal matter and that this stimulation should prove a definite advance in the treatment of articular diseases.

It is my belief that by the use of colloidal gold we are employing a substance which, as in the case of trypan blue, is deposited in various parts of the body, particularly the reticulo-endothelial system. The deposition of finely divided particles of gold in the histiocytes of the synovial membrane should result in the removal of products of inflammation, increase the defense mechanism in the synovial membrane, and likewise produce a stimulation of the general reticulo-endothelial system.¹¹

CONCLUSION

The conclusions derived from a review of the results obtained with gold salts and with the colloid of gold are as follows: Gold salts are more active therapeutically and likewise are more toxic. In view of the known clinical and biological action manifested by gold salts in the more severe forms of rheumatoid arthritis, they are probably superior to the colloid. On the other hand, the encouraging and apparently superior results obtained with the colloid, as compared with some of the older agents, especially in the less severe forms of rheumatoid arthritis and in fibrositis, are in support of the opinion that a therapeutic niche lying somewhere between the salt and some of these older and less spectacular agents should be conceded the colloid.

These results, of course, are not incontrovertible, but in view of the encouraging results obtained thus far by this study, and in view of the scarcity of actually desirable therapeutic agents in rheumatoid arthritis and other forms of infectious arthritis, colloid gold therapy should be given the opportunity of continued unbiased investigation. It might also appear that this form of therapy might be especially desirable in the treatment of generalized fibrositis with an infectious background, in the arthritides of known infectious origin, or in the incipient forms of rheumatoid arthritis.

SUMMARY

1. In view of the toxicity of gold salts in the treatment of rheumatoid arthritis which renders this type of therapy a definitely hazardous one, the therapeutic possibilities of some of the less soluble forms of gold were investigated. Gold in colloid form was chosen because of certain definite chemical considerations.

2. With the purpose in view of determining whether the beneficial attributes of gold were (something) inherent in the gold radical alone or whether these attributes were also demonstrable in the radicals of other heavy metals, platinum and bismuth were also employed.

3. Selenium, though not a heavy metal, because of its vaunted analgesic action in malignancies, was likewise used.

4. Platinum, bismuth, and selenium gave insufficient promise of therapeutic usefulness early in this clinical study and were, therefore, discarded.

5. Thirty-six patients in all were treated with the gold colloid over a period of two years. The colloid was given intramuscularly in individual and total doses equal to those I employed in a previous study with gold salts.

6. As a result of the present findings it is my opinion that in the gold colloid we are dealing with a more or less chemically inert form of gold, which, in contrast to gold salts, is only slightly soluble when injected in the body, and hence far less toxic.

7. The therapeutic results obtained with gold colloid may be partially explained on the basis of distribution of innumerable metallic particles throughout the reticulo-endothelial system, particularly in the deposition of these particles in the histiocytes of the synovial membrane, with the gradual liberation of gold ions and their consequent action on the body tissues.

8. No dramatic therapeutic qualities are espoused for colloidal gold in rheumatoid arthritis. In view of the beneficial response obtained in the present series, and because of the marked decrease in toxicity, it appears, however, that gold in colloid form is an agent worthy of further study and consideration for treatment of rheumatoid arthritis and generalized fibrositis with a toxic or infectious background.

I am indebted to E. A. H. King of the Crookes Laboratories, Inc., for his technical advice in preparing this paper.

REFERENCES

1. Tarsy, J. M.: Clinical Experiences With Gold Salts in the Treatment of Rheumatoid Arthritis, *New York State J. Med.* 40: 1185, 1940.
2. Karo, G.: Constitutional Treatment of Cancer, *Gac. méd. Espan.* 5: 23, 1930, *Abstr. Am. J. Cancer* 15: 2897, 1931.
3. Alpert, N.: Colloidal Metal in the Treatment of Malignancy, *Med. J. & Rec.* 132: Oct. 1, 347, 1930.
4. Karo, W.: Beitrag sur Behandlung, *Ztschr. f. Krebsforsch.* 32: 330, 1930; Inoperable Carcinoma, *Abstr. Am. J. Cancer* 15: 961, 1931.
5. Ochsner, E. H.: The Use of Colloidal Gold in Inoperable Cancer, *Internat. J. Med. & Surg.* 40: 100, 1927.
6. Ochsner, E. H.: Further Observations in the Use of Colloidal Gold in Inoperable Cancer, *Illinois M. J.* 50: 30, July, 1926.
7. Nowach, Louis H.: Papillary-Adeno Carcinoma of the Ovary, Report of Case Effectively Treated With Colloidal Gold, *Wisconsin M. J.* 25: 139, 1926.
8. Weiser, H. B.: *Inorganic Colloid Chemistry* 1: 134, 1933.
9. Watson-Williams, E.: Colloidal Selenium in Malignancy, *Brit. M. J.* 2: Oct. 11, 463, 1919.
10. Kuhns, J. G., and Weatherford, H. L.: Deposition of Colloidal and Particulate Matter in Articular Cavities, *Arch. Surg.* 33: 68, 1936.
11. Kling, David H.: The Mechanism of Gold Therapy in Rheumatoid Arthritis, *J. Lab. & Clin. Med.* 24: 1241, 1939.

THE RELATION OF THE NASOPHARYNX TO ULCERATIVE COLITIS*

PRELIMINARY REPORT

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A VOLUMINOUS literature has developed in the field of ulcerative colitis as to etiology and treatment. It is with full knowledge of these facts that we add our preliminary report of treatment of this condition.

Nonspecific ulcerative colitis is a chronic disease of unknown etiology, characterized by exacerbations and remissions, with symptomatology of bloody diarrhea and loss of weight, with proctoscopic findings of an edematous, granular, easily bleeding mucosa with ulcerations, either punctate or serpiginous; with blood findings of a secondary anemia, x-ray findings of the colon showing loss of haustrations and narrowing of the lumen. In these cases bacteriologic studies do not reveal any *amoeba histolytica*, *B. dysenteriae*, or diplostreptococci.

Our studies began four years ago when it became apparent to one of us (S.W.) as well as to others, notably Kunstler, Bargen, and Darrow, that exacerbations in ulcerative colitis were preceded very frequently by upper respiratory infections. We then began to study cultures taken from the nose, throat, and rectosigmoid mucosa of ulcerative colitis cases, and collected a series of 15 cases between 1934 and 1938.

The ages of these patients varied from 13 to 45 years, with typical histories of from five weeks' to eleven years' duration. Most of these patients had been under medical care before coming under our observation. The symptomatology included abdominal cramps, bloody diarrhea, loss of weight, weakness, and anemia. The proctoscopic findings showed granular mucosa, freely bleeding and ulcerated. Bacteriologic studies of the nose and throat revealed *staphylococcus albus*, 6 cases; hemolytic streptococcus, 4 cases; streptococci (nonhemolytic), 2 cases; *Streptococci viridans*, 1 case; *Staphylococci hemolyticus*, 1 case; *Micrococcus catarrhalis*, 1 case. Bacteriologic cultures taken directly from the mucosa of the rectosigmoid revealed *B. coli*, 9 cases; *Staphylococcus albus*, 5 cases; streptococci (nonhemolytic), 3 cases; *B. coli hemolyticus*, 2 cases; *Streptococci hemolyticus*, 1 case.

All patients except one were treated with a combined vaccine; this patient was given only a vaccine prepared from smears taken from the rectosigmoid. Vaccine from the nose and throat was added in this latter case, and after three injections of the combined vaccine, the bowel movements became formed and blood disappeared from the stools. These 15 patients have been well for a period ranging from nineteen months to four years.

*From the Department of Gastroenterology, New York Polyclinic Medical School and Hospital, Service of Dr. Samuel Weiss.

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Vaccines were prepared only from those organisms which were present simultaneously in the nose, throat, and rectosigmoid cultures. These vaccines were standardized to contain 1,000,000,000 organisms per cubic centimeter. Three dilutions were used, namely, 1:1, 1:10, and 1:100. We injected these vaccines three times a week in increasing doses from 0.1 c.c. to 1 c.c. for a total of 15 or more injections. Each week the three dilutions were used: the 1:100 the first day, the 1:10 the next injection, and the 1:1 dilution the last injection of that week. Each injection was increased by 0.1 c.c. each week.

The following is a case reported in detail because of the marked severity of the symptoms which improved after the combined vaccine therapy was instituted:

A young boy, 13 years of age, was admitted to the Polyclinie Hospital January 3, 1938, for the first time. For five weeks prior to his admission he had been having daily eight to ten loose stools, containing large amounts of bright red blood, severe generalized abdominal cramps, progressive loss of weight, and increased pallor. He also gave a history of staphylococcus infection of the eye five months previous which was treated successfully with a stock vaccine.

Physical examination revealed a pale, undernourished thin boy, weighing 108.5 pounds. Examination of the heart and lungs was negative. His nasopharynx showed no abnormality. His abdomen was markedly tender, especially in the left lower quadrant. There was no rigidity, and no masses were palpable.

Proctosigmoidoscopy performed on January 4, 1938, gave the following findings: The entire rectosigmoid mucosa showed marked redness with some false membrane formation. In places it was granular and there were numerous ulcerated areas, varying in size from a pin point to 1 cm. in diameter.

Cultures taken directly from the ulcerations showed the presence of many colonies of *B. coli* and *Staphylococcus albus*, but no streptococci. Cultures taken from the nasopharynx showed the presence of colonies of *Streptococcus viridans* and *Staphylococcus albus*. A vaccine was made of the *Staphylococcus albus*, this being the only organism present both in rectosigmoid and throat cultures.

Upon admission to the hospital the patient was given vaccine as well as other supportive therapy, such as liver extract injections, medicated enemas, and one transfusion. During the five weeks the patient remained at the hospital he failed to improve, the number of his bloody stools increased, and he had a further loss of weight, his lowest weight being 84 pounds. Following his dismissal he returned home where the vaccine therapy was continued. He also received several small transfusions at home. He began to improve about March 1, 1938, after having received 20 vaccine injections. Twelve more injections were given after this time. When seen on April 20, 1938, his weight was 104 pounds (a gain of 20 pounds in two months), and his general condition was very good. Bowel evacuations were normal.

Physical examination at this time was negative. The patient had no abdominal pain or discomfort: His hemoglobin was 68 per cent.

Proctosigmoidoscopy showed normal mucosa throughout. Cultures from the rectosigmoid mucosa showed the presence of *B. coli*, but no growth of *Staphylococcus albus*. Nose and throat cultures showed many colonies of *Streptococcus viridans* and a small number of *Staphylococcus albus*.

On September 8, 1938, his weight was 120 pounds; his hemoglobin was 78 per cent, his condition was excellent, and his bowel movements were normal.

The patient presented himself again on December 3, 1939. His health was still good. His weight was 137.5 pounds and his hemoglobin was 86 per cent. He claimed that this time he had one normally formed bowel movement daily.*

*Since presenting this paper the patient was seen several times thereafter.

COMMENT

What relation these various organisms from the nose, throat, and recto-sigmoid, as indicated in our studies, bear to the etiology of nonspecific ulcerative colitis we are not prepared to state. Whether by giving these various vaccines we are preventing upper respiratory infections, and in that way preventing exacerbations, we do not know. However, this seems to be the possible explanation of the rationale of our treatment. Nevertheless, we will continue to use this form of treatment, and hope that others will utilize it until such time as the specific etiology of nonspecific ulcerative colitis will be definitely established. We are of the opinion, as Paulson believes, that in those particular cases of ulcerative colitis, where amoeba, *B. dysenteriae*, and diplostreptococci of Barger are found, the resulting ulcerative colitis is secondary to these organisms.

CONCLUSIONS

We have discussed a series of cases of nonspecific ulcerative colitis presenting in detail one case. These cases were successfully treated with vaccines prepared from cultures from the nose, throat, and rectosigmoid containing only those organisms that were found simultaneously in the nasopharynx and rectosigmoid. We have had no recurrence in following these cases from a period of nineteen months to four years.

REFERENCES

1. Kunstler, M. B.: Medical Treatment of Ulcerative Colitis, *M. Rec.* 147: 200, 1938.
2. Winkelstein, Asher: Etiology and Therapy of Ulcerative Colitis, *Am. J. Digest. Dis. & Nutrition* 3: 839, 1937.
3. Fradkin, W. Z.: Bacteriological Aspects of Ulcerative Colitis, *New York State J. Med.* 37: 249, 1937.
4. Jelks, J. L.: Ulcerative Colitis Caused by Streptococcus, *Memphis M. J.* 12: 160, 1927.
5. Dack, G. M., Heinz, T. E., and Dragstedt, L. R.: Study of Bacteria in Ulcerative Colitis, *Arch. Surg.* 31: 225, 1935.
6. Osterlin, E. J., Johnson, A. W., Kinsey, J., and Willett, T.: Anti-Virus Treatment of Ulcerative Colitis, *Wisconsin M. J.* 34: 538, 1935.
7. Mackie, Thomas T.: Bacteriological and Clinical Study in Ulcerative Colitis, *Am. J. Digest. Dis. & Nutrition* 1: 466, 1934.
8. Barger, J. A.: Ten Years Treatment of Ulcerative Colitis, *Tr. Am. Gastro-Enterol. A.* 36: 49, 1933.
9. Garvin, J. D.: Relation of Focal Infection to Gastrointestinal Diseases, *Pennsylvania M. J.* 133: 616, 1930.
10. Paulson, M.: Present Status of Idiopathic Colitis, *J. A. M. A.* 101: 1687, 1933.
11. Darrow, C. H.: Oto-Laryngological Treatment of Chronic Ulcerative Colitis, *Ann. Oto., Rhin. & Laryng.* 40: 565, 1931.
12. Cook, Thomas J.: Focal Infection of Teeth and Elective Localization in Experimental Production, *J. Am. Dent. A.* 18: 2290, 1931.
13. Darrow, C. H.: Oto-Laryngological Treatment of Chronic Colitis, *Tr. Am. Laryng. Rhin. & Otol. Soc.* 37: 95, 1931.
14. Horster, H.: Torantil—Successful Therapy, *Arch. f. Verdauungskr.* 59: 301, 1936.
15. Weiss, Samuel: Secondary Infection in the Gastrointestinal Tract Following Oral Disease, *New York J. Dent.* 11: 126, 1941.

CLINICAL CHEMISTRY

THE EFFECTS OF INSULIN, METRAZOL, AND ELECTRIC SHOCK ON BLOOD PYRUVATE, LACTATE, AND GLUCOSE*

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IT HAS been observed by Rivers¹ that intravenous administration of large doses of vitamin B₁ early in stupor is often effective in preventing early convulsions with patients undergoing the insulin shock treatment for schizophrenia. Demole^{1a} had noted that administration of vitamin B₁ to rabbits during twenty-four hours previous to insulin injection usually prevented convulsions. These results suggested a possible correlation between the effects of insulin and other convulsive agents and the metabolism of carbohydrate via pyruvic acid, since it is well established that vitamin B₁, or rather the vitamin pyrophosphate, is concerned in pyruvic acid breakdown. A study has, therefore, been made of the changes in blood pyruvate, lactate, and glucose, which occur during insulin shock, after small insulin injections in normal subjects, and after metrazol and electric shock convulsions.

METHODS

Insulin was administered intramuscularly; metrazol and curare were administered intravenously. The electric shock treatment consisted in the brief application of alternating current at about 120 volts through electrodes applied to the sides of the forehead by a slight modification of the procedure of Cerletti and Bini.² Vitamin B₁ was injected intravenously.†

For each set of determinations about 10 c.c. of blood were drawn from the antecubital vein, the tourniquet being removed, directly into a test tube containing 40 mg. of potassium oxalate and 0.2 c.c. of 25 per cent sodium iodoacetate using a suction apparatus. Quick mixing with iodoacetate prevented the disappearance of pyruvate which otherwise occurs very rapidly.³ The presence of iodoacetate also prevented glycolytic increases of lactic acid. As soon as possible 2 c.c. of the sample were deproteinized with tungstic acid by the Haden modification of the Folin-Wu method, and 5 c.c. were deproteinized by running into 20 c.c. of 10 per cent trichloroacetic acid. All determinations were made in duplicate.

Glucose was determined on the tungstic filtrates by the method of Benedict, using the Klett-Summerson photoelectric colorimeter. With this colorimeter it was found that the rate of color development and the time for the color to

*From the Institute of the Pennsylvania Hospital, Philadelphia.
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†Vitamin B₁, thiamin chloride, was kindly supplied to T.D.R. by Eli Lilly and Company.

reach its maximum value depended upon the amount of glucose in the sample. In the usual method the solutions are cooled before adding the color reagent. Under these conditions, except with very small amounts of glucose, the color was still deepening rapidly ten minutes after adding the color reagent. Maximum color was generally reached, and readings could best be taken after fifteen to twenty-five minutes; after thirty minutes the color faded slowly.

In the presence of 0.05 per cent iodoacetate the color was found to develop more slowly; it never reached the correct value. However, if, instead of cooling the reaction mixture after the six-minute heating, the color reagent was added to the hot solution, the color developed rapidly. Under these conditions readings taken twenty minutes after adding the color reagent and diluting, gave correct values when compared with two standard solutions containing iodoacetate and run in the same manner.

Lactic acid was determined on the trichloroacetic acid filtrates, after removal of sugar with copper sulfate-copper hydroxide, by the method of Barker and Summerson.⁴ The presence of iodoacetate in the solutions to be analyzed had no effect on the results.

Pyruvic acid was determined on trichloroacetic acid filtrates by the method of Lu,⁵ as modified by Bueding and Wortis,³ the directions of the latter authors being followed exactly. Tungstic acid itself did not interfere with the determination of pure pyruvate solutions. But tungstic acid filtrates from blood could not be used for the pyruvate determinations, since low values were always obtained and, when known amounts of pyruvate were added to blood before deproteinizing with tungstic acid, the recovery was low. Evidently the protein precipitated with tungstic acid carries down some pyruvate. Complete recovery was obtained after trichloroacetic acid precipitation. The trichloroacetic filtrates could be kept for several days before analysis without appreciable change. Oxalate and iodoacetate did not interfere with the determination. While the method is more specific than the Clift and Cook⁶ determination of bisulfite-binding substances and gives much lower values with blood,⁷ it is not completely specific. It has been assumed in this paper that pyruvate itself is largely responsible for the values obtained, but it must be remembered that α -ketoglutarate and oxaloacetate, and possibly other substances, are included in the values.

RESULTS

The results of all experiments are presented diagrammatically in Figs. 1 to 17. The ordinates for lactic acid and pyruvic acid are respectively twice and twenty times the size of those for glucose.

The legends for the figures describe the material for the various experiments. All persons were initially fasting, except those represented in Figs. 16 and 17. As far as possible persons were initially resting, but the condition of the patients did not always allow this condition to be well controlled.

Lactate.—No consistent changes in the amount of lactate in the blood during insulin stupor were observed, but some fluctuations occurred which could probably be attributed to variations in muscular activity of the patients. In three of four cases of resting normal persons receiving small doses of insulin

(see Figs. 5, 7, 8), slight rises in blood lactate occurred, possibly as a result of extra visceral muscular activity due to the hypoglycemia. Rises of blood lactate in experimental animals treated with insulin have been observed and attributed to increased muscular activity by earlier workers.⁸

Metrazol and electric shock convulsions caused extremely large rises in blood lactate,⁹⁻¹¹ obviously as a result of the intense muscular contractions. The rapidity of the rise after electric shock convulsions is noteworthy. Possibly the same occurs during metrazol convulsions, but blood could not be drawn as immediately after metrazol as after electric shock. When the muscular activity was moderated by previous administration of curare, the rise in blood lactate was less marked.

Pyruvate.—Von Euler and Högborg¹² have stated that an hour after administration of 10 units of insulin to normal persons, decreases of blood pyruvate of 75 to 100 per cent were found. Though we used a similar method for pyruvate determination, we never found any appreciable decrease in blood pyruvate as a result of administration of small or large doses of insulin.

Fluctuations in blood pyruvate followed closely the direction of the fluctuations in blood lactate, and it seems clear that the amounts of these two substances are related to each other and are affected similarly by muscular activity and not specifically by insulin or the other agents. The fluctuations in pyruvate, however, were always much less wide than those of lactate. Johnson and Edwards¹³ found increases of lactate and pyruvate in blood and urine with young men after severe exercise and suggested that the pyruvate was produced, in association with lactate, by the active muscles; this suggestion was shown to be true by Bollman and Flock¹⁴ in experiments with exercising rat muscles.

It is reasonable to believe that, with large doses of insulin, consciousness is lost and other brain functions are affected as a result of a lack of oxidizable substrate (glucose) for the oxidations necessary to maintain brain activity.¹⁵ However, no correlation was observed between the state of consciousness and the level of blood pyruvate or lactate during insulin or the other treatments. Wortis and Goldfarb¹⁶ observed that injections of lactate into patients in insulin coma had little effect on the oxygen consumption of the brain and did not arouse the patient. Mann and Magath¹⁷ were unable to relieve the convulsions of hepatectomized animals with sodium lactate. With hepatectomized abdominal eviscerated rabbits, Maddock and co-workers¹⁸ found that the cortical electrical potentials could be restored by intravenous injections of glucose but not of a number of other substances including pyruvate. With patients in insulin hypoglycemia Himwich and associates¹⁹ found no significant absorption of lactic acid from the blood circulating through the brain, and Wortis and Goldfarb²⁰ found no sign of metabolism of injected lactate or pyruvate by the brain. It thus seems that lactate and pyruvate in the blood are not available for oxidations in the brain, although the brain is well known to oxidize these substances *in vitro*.

Injections of large doses of vitamin B₁ into patients in insulin stupor produced no definite effect on blood pyruvate or glucose* within the period of

*Demole²¹ found that the previous administration of the vitamin to rabbits often diminished the hypoglycemia caused by insulin.

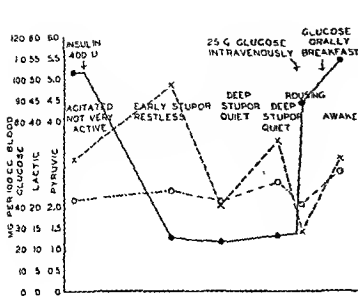


FIG 1

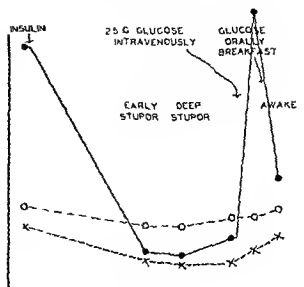


FIG 2

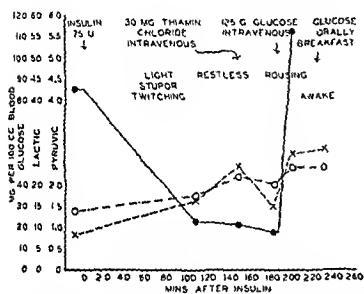


FIG 3

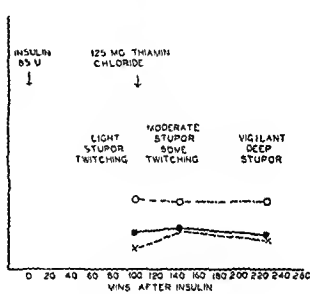


FIG 4

Insulin shock treatments.

Glucose —•—•, Lactate x---x, Pyruvate o---o.

Fig. 1.—L. K., male, 20 years old, paranoid schizophrenic.

Fig. 2.—N. N., male, 34 years old, paranoid schizophrenic.

Fig. 3.—G. W., female, 22 years old, paranoid schizophrenic.

Fig. 4.—J. I., male, 15 years old, paranoid schizophrenic.

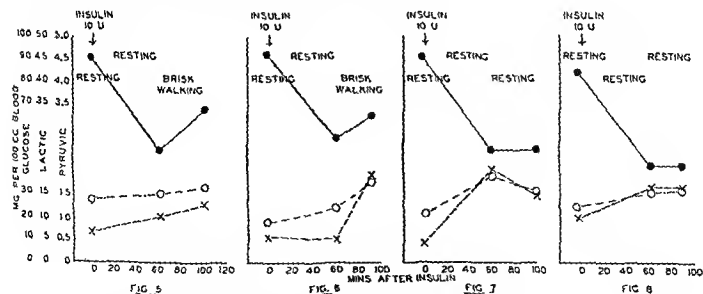


FIG 5

FIG 6

FIG 7

FIG 8

Insulin in small doses to normal subjects.

Glucose —•—•, Lactate x---x, Pyruvate o---o.

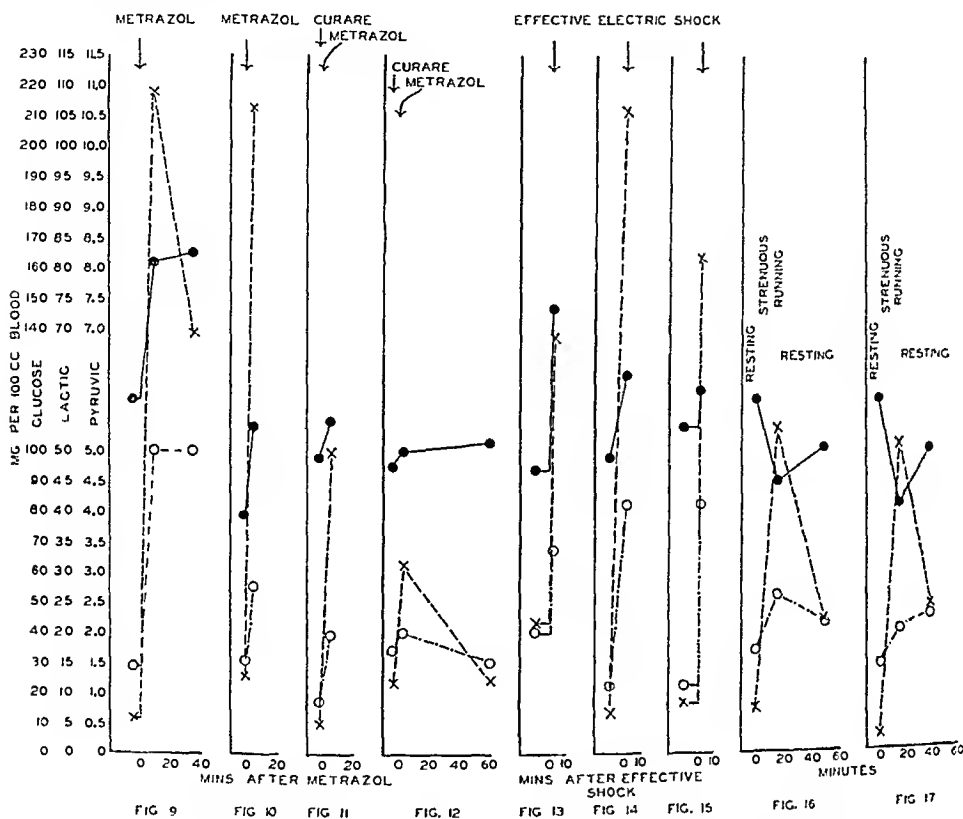
Fig. 5.—A. E., male, aged 37 years.

Fig. 6.—B. L., male, aged 24 years.

Fig. 7.—F. E., female, aged 34 years.

Fig. 8.—B. P., female, aged 20 years.

the experiment. Platt and Lu⁷ found a lag period of several hours before the high blood pyruvate of vitamin B₁-deficient patients was affected by curative small doses (5 mg.) of the vitamin. The observation of Rivers¹ on the effect of vitamin B₁ on insulin convulsions remains unexplained by the present work. A possible explanation is that during insulin hypoglycemia slight available amounts of glucose continue to be metabolized by the brain, producing within the cells suboptimal amounts of intermediary metabolites, the oxidation of which can be sufficiently stimulated by excess vitamin B₁ to produce more normal conditions.



Metrazol convulsions with (Figs. 11 and 12) and without (Figs. 9 and 10) partial curarization.

Glucose ●—●, Lactate x—x, Pyruvate o—o.

- Fig. 9.—G. L., female, aged 53 years, involuntional melancholia.
 Fig. 10.—M. R., female, aged 40 years, involuntional melancholia.
 Fig. 11.—V. H., male, aged 50 years, involuntional melancholia.
 Fig. 12.—R. H., male, aged 54 years, involuntional melancholia.

Electric shock.

Glucose ●—●, Lactate x—x, Pyruvate o—o.

- Fig. 13.—F. K., female, 48 years old, involuntional melancholia.
 Fig. 14.—Z. S., female, 64 years old, manic-depressive depressed.
 Fig. 15.—R. M., female, 52 years old, involuntional melancholia.

Brief exercise to exhaustion on normal subjects.

Glucose ●—●, Lactate x—x, Pyruvate o—o.

- Fig. 16.—B. P., female, 20 years old. About two hours after lunch.
 Fig. 17.—F. E., female, 34 years old. About two hours after lunch.

Bueding and Wortis³ found that the pyruvic acid content of normal blood fell in the range 0.77 to 1.16 mg. per 100 c.c., averaging 0.98. It will be noticed that our initial resting figures are mostly appreciably higher, 0.9 to 2.2, averaging 1.4. The difference may be due to the fact that Bueding and Wortis' subjects were probably more thoroughly rested than ours.

Glucose.—Short severe muscular effort is well known to raise the fasting blood sugar.²¹ Blood glucose rose rapidly and considerably in metrazol (see Meduna and Rohny;¹¹ Loman and co-workers;²² and Redlich²³) and electric shock convulsions. Exercise after small doses of insulin to normal persons also raised the blood sugar considerably (Figs. 5 and 6). During brief exhausting exercise of nonfasting normal persons, the blood sugar fell steeply and recovered during rest (Figs. 16 and 17).

SUMMARY

Neither insulin in small or large doses, nor metrazol or electric shock treatments, have any specific effect on the level of blood pyruvate, and probably none on the lactate level, in human beings. The concentration of these substances bore no relation to the blood sugar level nor to the condition of brain functions.

Blood pyruvate and lactate rose and fell together, and the rises seemed to be the result only of muscular activity. In metrazol and electric shock the rises of both substances, and of blood glucose, were very high; curare decreased the effect of metrazol convulsions on blood lactate, pyruvate, and glucose. The changes in blood pyruvate were less marked than those of lactate.

REFERENCES

1. Rivers, T. D.: Effect of Vitamin B₁, Sucrose and Sorbitol on Certain Complications of Insulin Hypoglycemia: A Clinical Report, *Arch. Neurol. & Psychiat.* 44: 910, 1940.
- 1a. Demole, V.: Dementia Praecox, Hypoglycemia, Insulin, Vitamin B₁, *Proceedings of the 87th Meeting of the Swiss Psychiatric Association at Munsingen, Berne, Am. J. Psychiat. Suppl.* 94: 59, 1937.
2. Cerletti, U., and Bini, L.: L'Elettroshock, *Arch. gen. di neurol., psichiat. e psicoanal.* 19: 267, 1938.
3. Rueding, E., and Wartis, H.: Stabilization and Determination of Pyruvic Acid in the Blood, *J. Biol. Chem.* 133: 585, 1940.
4. Barker, S. B., and Summerson, W. H.: The Colorimetric Determination of Lactic Acid in Biological Material, *J. Biol. Chem.* 138: 535, 1941.
5. Lu, G. D.: Studies on the Metabolism of Pyruvic Acid in Normal and Vitamin B₁ Deficient States: I. A Rapid, Specific and Sensitive Method for the Estimation of Blood Pyruvate, *Biochem. J.* 33: 249, 1939.
6. Cliff, F. P., and Cook, R. P.: A Method of Determination of Some Biologically Important Aldehydes and Ketones, With Special Reference to Pyruvic Acid and Methylglyoxal, *Biochem. J.* 26: 1788, 1932.
7. Platt, B. S., and Lu, G. D.: Studies on the Metabolism of Pyruvic Acid and Other Carbonyl Compounds in Beri-Beri and the Effect of Vitamin B₁, *Biochem. J.* 33: 1525, 1939.
8. Briggs, A. P., Koehlig, I., Doisy, E. A., and Weber, C. J.: Some Changes in the Composition of the Blood Due to the Injection of Insulin, *J. Biol. Chem.* 58: 721, 1924.
- Best, C. H., and Ridout, J. H.: Observations on Blood Lactic Acid After Insulin, *J. Biol. Chem.* 63: 197, 1935.
- Cori, C. F.: The Influence of Insulin and Epinephrine on the Lactic Acid Content of Blood and Tissues, *J. Biol. Chem.* 63: 253, 1925.
9. Humrich, H. E., Bowman, K. M., Fazekas, J. F., and Orenstein, L. L.: Effect of Metrazol Convulsions on Brain Metabolism, *Proc. Soc. Exper. Biol. & Med.* 37: 359, 1937.
10. Katzenellenbogen, S., Brody, M. W., Hayman, M., and Margolin, E.: Metrazol Convulsions in Man, *Am. J. Psychiat.* 95: 1343, 1939.
11. Meduna, L., and Rohny, B.: Insulin and Metrazol Treatment of Schizophrenia, *Lancet* 236: 1139, 1939; *Chem. Abst.* 34: 5166, 1940.
12. von Euler, H., and Högberg, B.: Vitamin-B₁, Insulin und Phosphatstoffwechsel, *Naturwissenschaften* 28: 29, 1940.
- Zur Kenntnis der Vitamin-B₁ Wirkungen, *Ztschr. f. physiol. Chem.* 263: 49, 1940.
13. Johnson, R. E., and Edwards, H. T.: Lactate and Pyruvate in Blood and Urine After Exercise, *J. Biol. Chem.* 118: 427, 1937.

14. Bollman, J. L., and Flock, E. V.: Pyruvate in Working Muscles of Normal and Vitamin B₁₂-Deficient Rats, *J. Biol. Chem.* 130: 565, 1939.
15. Himwich, H. E., Bowman, K. M., Wortis, J., and Fazekas, J. F.: Brain Metabolism During Hypoglycemic Treatment of Schizophrenia, *Science* 86: 271, 1937.
Metabolism of the Brain During Insulin and Metrazol Treatments of Schizophrenia, *J. A. M. A.* 112: 1572, 1939.
- Himwich, H. E., Bowman, K. M., Fazekas, J. F., and Orenstein, L. L.: Effect of Metrazol Convulsions on Brain Metabolism, *Proc. Soc. Exper. Biol. & Med.* 37: 359, 1937.
- Himwich, H. E., Frostig, J. P., Fazekas, J. F., and Hadidian, Z.: The Mechanism of the Symptoms of Insulin Hypoglycemia, *Am. J. Psychiat.* 96: 371, 1939.
- Himwich, H. E., Frostig, J. P., Fazekas, J. F., Hadidian, Z., and Hoagland, H.: Clinical, Electroencephalographic, and Biochemical Changes During Insulin Hypoglycemia, *Proc. Soc. Exper. Biol. & Med.* 40: 401, 1939.
16. Wortis, J., and Goldfarb, W.: The Availability of Sodium Lactate for Brain Oxidations, *Am. J. Physiol.* 129: 502, 1940.
17. Mann, F. C., and Magath, T. B.: Studies on the Physiology of the Liver. III. The Effect of Administration of Glucose in the Condition Following Total Extirpation of the Liver, *Arch. Int. Med.* 30: 171, 1922.
18. Maddock, S., Hawkins, J. E., Jr., and Holmes, E. G.: The Inadequacy of Substances of the "Glucose Cycle" for Maintenance of Normal Cortical Potentials During Hypoglycemia Produced by Hepatectomy With Abdominal Evisceration, *Am. J. Physiol.* 125: 551, 1939.
19. Himwich, H. E., Bowman, K. M., Wortis, J., and Fazekas, J. F.: Biochemical Changes Occurring in the Cerebral Blood During the Insulin Treatment of Schizophrenia, *J. Nerv. & Ment. Dis.* 89: 273, 1939.
20. Wortis, J., and Goldfarb, W.: A Method of Studying the Availability of Various Substrates for Human Brain Metabolism During Therapeutic Insulin Shock, *Science* 91: 270, 1940.
21. Dill, D. B., Edwards, H. T., and Mead, S.: Blood Sugar Regulation in Exercise, *Am. J. Physiol.* 111: 21, 1935.
22. Loman, J., Rinkel, M., and Myerson, A.: Metrazol Convulsions. Changes in Oxygen, Carbon Dioxide, and Sugar Content of Arterial and Internal Jugular Venous Blood, *Arch. Neurol. & Psychiat.* 43: 682, 1940.
23. Redlich, H. C.: Metrazol Shock Treatment. Pharmacological and Biochemical Studies, *Am. J. Psychiat.* 96: 193, 1939.

BLOOD SPECIFIC GRAVITY STUDIES*

RELATIONSHIP OF SPECIFIC GRAVITY OF WHOLE BLOOD TO SPECIFIC GRAVITY OF PLASMA, RED BLOOD CELL COUNT, HEMATOCRIT, AND HEMOGLOBIN AS INDICATORS OF HEMOCONCENTRATION*

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THERE has been an increasing familiarity with specific gravity studies, red blood cell counts, hemoglobin determinations, and hematocrit studies as methods of measuring hemoconcentration and hemodilution in shock and related conditions. Many investigators feel that specific gravity study is the simplest and most practical method of recognizing and following hemoconcentration during the course and treatment of shock. The method of Barbour and Hamilton¹ has placed this means of study on a practical accurate basis, and Seudder² has popularized the method in the study of shock. In a previous publication,³ the specific gravity method has been demonstrated to be not only an excellent method of studying the course of hemoconcentration during experi-

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mental shock, but also a means of recognizing relative blood volume changes. This latter application depends upon the assumption of a relatively constant erythrocyte specific gravity. In a series of experiments on dogs in the study of shock, it was found that the erythrocyte specific gravity varied only slightly in normal animals and during the course of shock.

It has been felt necessary to investigate the erythrocyte specific gravity in human beings and to determine whether this factor is as nearly constant as it is in dogs. In this study also particular attention has been paid to the linear relationship between the various methods of indicating hemoconcentration, as well as the relative efficacy of each of these methods.

METHODS

A series of 20 normal persons with red blood cell count over 4,000,000 and a series of 10 patients, most of whom had some form of anemia, were studied to determine the specific gravity of the red blood cells. Blood was obtained by venepuncture with minimal stasis. Heparin was used as the anti-coagulant. On each sample, red blood cell counts, hemoglobin by Sanford and Sheard method¹ using a photoelectric colorimeter, hematocrit using Wintrobe tubes,⁵ and specific gravity of whole blood and plasma using the method of Barbour and Hamilton¹ were carried out. Mean cell volume, mean cell hemoglobin, and mean cell hemoglobin concentration were calculated from the red blood cell count, hemoglobin, and hematocrit. The specific gravity of the red blood cells was calculated as follows:¹

Sp. gr. of R. B. C. =

$$\frac{(\text{Sp. gr. whole blood} \times 100) - \{\text{Sp. gr. plasma} \times (100 - \text{Hematocrit})\}}{\text{Hematocrit}}$$

The efficacy of protein concentration as an indicator of hemoconcentration was studied in a series of dogs in which shock was experimentally produced during which hemoconcentration studies were carried out.

RESULTS

Table I shows the results of the studies on human beings. The specific gravity of red blood cells shows only minor variation in normal human beings, varying from 1.0936 to 1.0998, with an average of 1.0971. In patients with anemia, however, the variation was somewhat greater, from 1.0868 to 1.1045, this variation bearing slight but not absolute relationship to the mean cell hemoglobin concentration. It would appear that a close relationship between mean cell hemoglobin concentration and specific gravity of red blood cells exists, and the absence of such may be due to technical variations. It would seem that, although in anemia and other abnormal conditions, the red blood cell specific gravity may vary from the average, in the same person the factor is sufficiently constant that the specific gravity method is an accurate means of indicating relative blood volume changes. A study of the literature shows that Oestreich⁶ in 1931 studied the specific gravity of blood using methods susceptible to serious criticism; he found the specific gravity of erythrocytes around 1.09, a somewhat lower figure than that obtained here.

TABLE I

BLOOD STUDIES ON NORMAL PERSONS, ANEMIC PATIENTS, PATIENTS WITH MILD IRON DEFICIENCY ANEMIA, AND ONE WITH POLYCYTHEMIA VERA

	R.B.C. COUNT IN MILLIONS	HEMO- GLOBIN GM./100 C.C.	HEMATO- CRIT	MCV	MCH	MCHC	SP. GR. PLASMA	SP. GR. BLOOD	SP. GR. ERYTH- ROCYTES
Normal	5.07	15.85	44.0	86.8	31.3	36.0	1.0264	1.0560	1.0936
Normal	4.76	14.15	47.0	98.7	29.7	30.1	1.0252	1.0582	1.0953
Normal	4.85	15.85	45.5	93.8	32.7	35.6	1.0264	1.0571	1.0954
Normal	4.60	15.85	43.0	93.5	34.4	36.9	1.0257	1.0557	1.0956
Normal	5.38	16.10	47.5	88.3	29.9	33.9	1.0251	1.0589	1.0962
Normal	4.97	16.65	46.8	94.2	33.5	35.6	1.0257	1.0588	1.0964
Normal	5.50	16.90	47.5	86.4	30.7	35.6	1.0249	1.0589	1.0965
Normal	4.64	14.70	43.0	92.7	31.7	34.2	1.0278	1.0573	1.0965
Normal	4.22	14.15	39.5	93.6	33.5	35.8	1.0253	1.0534	1.0965
Normal	4.30	14.60	39.5	91.9	34.0	37.0	1.0251	1.0533	1.0965
Normal	4.84	14.70	42.0	86.7	30.4	35.0	1.0260	1.0559	1.0971
Normal	4.47	14.45	39.0	89.2	32.3	37.1	1.0266	1.0542	1.0974
Normal	5.53	15.85	46.0	83.2	28.7	34.5	1.0266	1.0594	1.0979
Normal	4.06	14.15	38.0	93.6	34.9	37.3	1.0272	1.0541	1.0979
Normal	5.09	16.10	45.0	88.4	31.6	35.6	1.0260	1.0584	1.0980
Normal	5.13	15.55	43.5	84.8	30.3	35.1	1.0250	1.0569	1.0984
Normal	5.08	15.55	47.5	83.5	30.6	32.7	1.0293	1.0622	1.0986
Normal	4.86	14.15	39.0	80.2	29.1	36.3	1.0240	1.0533	1.0992
Normal	5.77	17.40	48.0	83.2	30.2	36.3	1.0276	1.0622	1.0998
Normal	5.09	15.55	47.0	92.3	30.6	33.1	1.0258	1.0603	1.0993
Patient	3.70	9.15	31.0	83.8	24.7	29.5	1.0251	1.0442	1.0868
Patient	2.53	8.20	29.0	114.6	32.4	28.3	1.0246	1.0433	1.0891
Patient	8.52	25.80	78.0	91.5	30.3	33.1	1.0269	1.0758	1.0896
Patient	4.20	13.25	36.5	96.1	34.9	36.5	1.0251	1.0521	1.0911
Patient	4.85	13.35	45.5	93.8	28.5	30.5	1.0279	1.0569	1.0916
Patient	4.43	12.90	38.5	86.9	29.0	33.5	1.0247	1.0521	1.0958
Patient	4.42	12.60	37.1	83.9	28.5	33.9	1.0276	1.0535	1.0973
Patient	4.02	13.50	38.5	83.5	29.3	35.1	1.0259	1.0542	1.0995
Patient	4.70	15.3	41.7	88.7	32.6	36.7	1.0263	1.0580	1.1024
Patient	3.36	11.7	32.9	97.9	34.8	35.6	1.0263	1.0470	1.1045

With the average specific gravity of human erythrocytes known, the hematocrit may be calculated from the specific gravity of whole blood and plasma as follows:

$$\text{Hematocrit} = \frac{(\text{Sp. gr. whole blood} \times 100) - [\text{Sp. gr.} \times (100 - \text{Hematocrit})]}{1.0971}$$

The relationship between specific gravity of whole blood and the hematocrit, red blood cell count, and hemoglobin would be expected to be fairly constant. Since, however, the specific gravity of whole blood is partly dependent upon the specific gravity of the plasma, there should be some departure from this relationship when this latter factor is not taken into account. For practical purposes, in spite of this, the hematocrit, red blood cell count, and hemoglobin bear a distinct mathematical relationship to, and can be fairly accurately estimated from, the specific gravity of the whole blood. The relationship between these is shown in Figs. 1, 2, 3. This relationship between specific gravity of whole blood and the hematocrit, hemoglobin, and red cell count may be expressed as follows:

$$(1) \text{Hematocrit} = (\text{Sp. gr. whole blood} - 1.0457) \times 1,000 + 32.$$

$$(2) \text{Hemoglobin} = (\text{Sp. gr. whole blood} - 1.0470) \times 386 + 11.$$

$$(3) \text{Red blood cell count in millions} = (\text{Sp. gr. whole blood} - 1.0466) \times 14.5 \div 3.5.$$

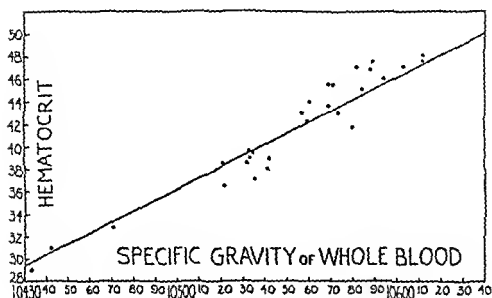


Fig. 1.

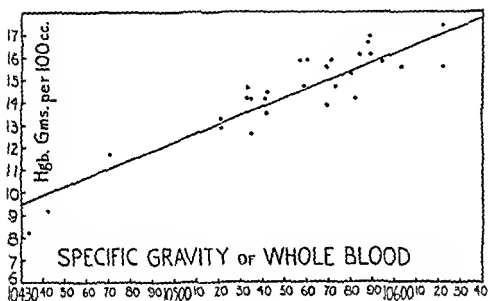


Fig. 2.

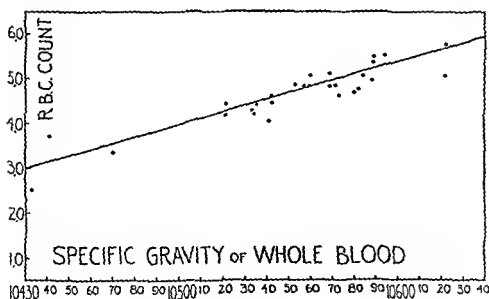


Fig. 3.

Naturally these equations will yield results slightly in error of the actual values, but these variations do not exceed the limits of technical error by any appreciable extent. Because of this rather accurate correlation between the specific gravity of whole blood and hematocrit, hemoglobin, and red blood cell count, it would seem that they are more or less uniformly efficacious as indicators of hemoconcentration. The value of these studies of peripheral blood as measures of hemoconcentration and relative blood volume change has been further accentuated by recent studies of Ebert and Stead⁷ which showed the relative constancy of circulating red blood cell volume in human beings under various circumstances.

TABLE II

HEMOCONCENTRATION STUDIES IN DOGS IN WHICH SHOCK WAS INDUCED BY INTRAPERITONEAL INJECTION OF HYPERTONIC SALINE (25%)

DOG		R.B.C. COUNT IN MIL- LIONS	HEMO- GLOBIN GM./100 C.C.	HEM- ATO- CRIT	PRO- TEIN GM./100 C.C.	SP. GR. BLOOD	PRO- TEIN	HEM- ATO- CRIT	HEMO- GLO- BIN	R.B.C. COUNT
		PERCENTAGE INCREASE								
1	Control	4.2	10.15	33	5.37	1.0465				
	Shock	9.64	20.15	60.5	6.09	1.0691	13.4	83.3	98	127
2	Control	4.76	12.45	32	5.58	1.0529				
	Shock	10.74	24.0	61.5	6.53	1.0817	17	92	92.8	125
3	Control	5.16	12.95	38.5	6.12	1.0509				
	Shock	8.43	18.9	56.5	5.85	1.0649	-5	46.7	46.2	63.3
4	Control	5.03	12.35	36.0	6.56	1.0500				
	Shock	7.95	17.9	51.5	6.7	1.0642	3.6	43	45	58
5	Control	6.37	17.15	48.5	6.39	1.0610				
	Shock	9.63	23.15	70	7.28	1.0749	14.1	44.3	35	51

Table II represents hemoconcentration studies in five dogs in which shock was induced by intraperitoneal injection of hypertonic saline. The percentage increase of hemoglobin, hematocrit, and red blood cell count from control studies to shock values demonstrates the relative efficiency of each method of determining hemoconcentration. It will be seen that fairly accurate correlation in the values was obtained. Protein concentration was estimated from the specific gravity of the plasma according to the method of Weech, Reeves, and Goettseh.⁸ A comparison of the percentage change of protein concentration from control to shock values with corresponding values of other measures of hemoconcentration shows the lack of efficiency of protein concentration as a measure of hemoconcentration or relative blood volume change. This is to be expected, since proteins are lost from the circulating blood during the development of shock.

SUMMARY

1. The average specific gravity of normal human erythrocytes is 1.0971. This factor is fairly constant in different persons, but in anemia there frequently is a greater departure from the average than in normals.

2. The hematocrit may be calculated from the specific gravity of whole blood and of plasma in human beings. Also a fairly accurate estimate of red blood cell count, hemoglobin, and hematocrit may be made from the specific gravity of whole blood alone, since these values bear a constant relationship to one another.

3. Specific gravity of whole blood, hemoglobin concentration, hematocrit, and red blood cell count are uniformly efficacious as indicators of hemoconcentration and relative blood volume change.

4. Protein concentration or specific gravity of plasma is not a satisfactory indicator of hemoconcentration during the course of shock, since protein is lost from the circulating blood.

REFERENCES

1. Barbour, H. G., and Hamilton, W. F.: The Falling Drop Method for Determining Specific Gravity, *J. Biol. Chem.* 69: 625, 1926.
2. Scudder, John: Shock. Blood Studies as a Guide to Therapy, Philadelphia, 1940, J. B. Lippincott Co.
3. Ashworth, C. T., and Tigertt, W. D.: A Simple Rapid Method for Determining Relative Blood Volume Changes by Specific Gravity Studies, *J. LAB. & CLIN. MED.* 26: 1545, 1941.
4. Sanford, A. H., and Sheard, C.: The Determination of Hemoglobin With the Photo-Electrometer, *J. LAB. & CLIN. MED.* 15: 483, 1930.
5. Wintrobe, M. M.: A Simple and Accurate Hematocrit, *J. LAB. & CLIN. MED.* 15: 287, 1929.
6. Oestreich, C.: Ueber das spezifische Gewicht des Blutes und seiner Bestandteile, *Klin. Wchnschr.* 10: 160, 1931.
7. Ebert, R. V., and Stead, E. A.: Demonstration That in Normal Man No Reserves of Blood Are Mobilized by Exercise, Epinephrine, and Hemorrhage, *Am. J. M. Sc.* 201: 655, 1941.
8. Weech, A. A., Reeves, E. B., and Goettsch, E.: The Relationship Between Specific Gravity and Protein Content in Plasma, Serum and Transudate From Dogs, *J. Biol. Chem.* 113: 167, 1936.

INDOLURIA IN RHEUMATOID ARTHRITIS*

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INDOLE is formed by bacterial action upon protein. This action may take place in the gastrointestinal tract, the urinary tract, or in some focus of infection.³ Normally, indole is oxidized in the body to indoxyl, which is conjugated with potassium hydrogen sulfate, with the formation of indican that is excreted in the urine. The indoxyl detoxifying mechanism is located in the liver.

In normal persons under normal conditions, free indole does not appear in the urine. In over 100 determinations on normal persons, urinary indole was found in only one, and then only upon one occasion.⁴ On the other hand, indoluria was encountered in various pathologic conditions.^{2, 4}

Vaughan¹¹ was unable to detect the presence of indole in specimens of urine from diseased persons. However, Vaughan,¹² and Carnes and Lewis,³ point to the possibility that the urine may contain a substance that might be converted into indole by the action of *E. coli*. These investigators observed that indole-negative urines may become positive upon standing.

Deficient oxidation, impaired liver function, sulfur deficiency, excessive indole production in the intestinal tract, and many other concepts were advanced as theoretical possible causes for the appearance of free indole in the urine.^{2, 4}

*This study was conducted from February 2 to July 15, 1938.

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Forbes and Neale⁴ reported indoluria in a variety of pathologic conditions, including rheumatic fever, gonococcal, infectious, rheumatoid, and hypertrophic arthritis. These investigators used steam distillation in the presence of tartaric acid, followed by colorimetry, to determine quantitatively the indole in the urine. Values less than 0.05 mg. per liter of urine were regarded as a trace. Most cases ran from 0.5 to 1.5 mg. per liter of urine.

As indoluria might be evidence of the liver failing in its detoxifying action, possibly because of sulfur deficiency, it seemed logical to Forbes and Neale and their collaborators⁵ to give patients with chronic arthritis a diet that contained a high proportion of sulfur amino acids. It was noted that clinical improvement was accompanied by a decrease and eventual disappearance of the indoluria.

In contrast, it seemed improbable to Basinski and Lewis² that indoluria should be related to a lack of sulfur needed for conjugation. In their patients having indoluria, the cystine level in the fingernails was found to be normal.

The demonstration of indoluria in patients with rheumatoid arthritis by Forbes and Neale,⁴ prompted me to test the urines of 88 patients with rheumatoid arthritis for the presence of free indole. The patients, consisting of 46 females and 42 males, were partly from my private practice, partly from the People's Hospital at Pistany Spa, Czechoslovakia. This hospital, equipped with 600 beds, is dedicated to the treatment of panel patients suffering from chronic arthritis and other rheumatoid conditions. The patients stayed three to four weeks at the hospital, the average duration of the "spa cure." The spa treatment consists of the use of naturally hot sulfur springs and of the sulfur-containing mud of volcanic origin, the latter being administered in the form of mud baths and packs. The rationale and technique of the spa treatment were described in a previous article.¹⁰

Considering that sulfur is absorbed through the skin,^{3, 7, 8} and that its absorption had been demonstrated from the mud applied to the shaved skin of a rabbit,⁶ I attempted to investigate not only the presence of free indole in the urines of patients with rheumatoid arthritis, but also its fate in the urine of patients who take a "spa cure." However, I was unable to complete the latter part of my study.

The samples of urine, obtained from the patients at any hour throughout the day, and at any period in the course of the spa treatment, were collected as follows: After the glans penis and the urethral orifice of the male patient had been washed, the urine was collected in two portions. The first specimen of urine was discarded, the second one was collected in a sterile container and promptly tested for indole. The female patients cleansed their external genitals, and voided into two containers, the second of which was sterile and its contents were used for indole determination, which analysis was made within thirty to sixty minutes. The method of analysis used by Forbes and Neale⁴ was employed in the study on indoluria.

The analytical results of the examined urine specimens obtained from 88 patients with rheumatoid arthritis were as follows: In the 46 females, the values of indole were between 0.3 and 1.8 mg. per liter of urine; in the 42

males, the indole values were between 0.2 and 1.94 mg. per liter of urine. Less than 1 mg. of indole per liter of urine was found in 32 females (69.6 per cent), and in 31 males (73.8 per cent). More than 1 mg. of indole per liter of urine was found in 14 females (30.4 per cent) and in 11 males (26.2 per cent).

The results reported here are in agreement with those of Neale⁹ who, recently, in a series of 50 cases of proliferative arthritis in female patients obtained urine aseptically by catheterization and demonstrated free indole in urine which was shown to be sterile by culture. This indoluria disappeared when the patients responded to treatment.

SUMMARY

Free indole was shown to be present in the freshly voided urine samples obtained from 88 patients with rheumatoid arthritis. These results confirm the observations reported by Forbes and Neale.⁴

I wish to acknowledge the cooperation of Dr. Anna Igumova, Head of the Chemical Research Laboratory at Pistany Spa. I gratefully offer an expression of appreciation to her.

REFERENCES

1. Basch, F.: Ueber Schwefelwasserstoffvergiftung bei ausserlicher Applikation von elementarem Schwefel in Salbenform, *Arch. f. exper. Path. u. Pharmacol.* 111: 120, 1926.
2. Basinski, D. H., and Lewis, G. T.: Indoluria and Its Relation to Sulfur Deficiency, *Proc. Soc. Exper. Biol. & Med.* 42: 628, 1939.
3. Carnes, H. E., and Lewis, G. T.: The Sources of Urinary Indol, *J. LAB. & CLIN. MED.* 23: 459, 1938.
4. Forbes, J. C., and Neale, R. C.: Studies on Indoluria, *J. LAB. & CLIN. MED.* 20: 1017, 1935.
5. Forbes, J. C., Neale, R. C., Hile, O. L., Armistead, D. B., and Rucker, S. L.: Studies on the Effect of a High Sulphur Low Carbohydrate Diet in Chronic Arthritis, *J. LAB. & CLIN. MED.* 21: 1036, 1936.
6. Kilian, V.: Beitrag zur Frage der Chemischen Grundlagen der Schlammtherapie, *Arch. f. exper. Path. u. Pharmacol.* 178: 197, 1935.
7. Maliva, E.: Beitrage zur Experimentellen Balneologie. I. Ist eine Schwefelresorption durch die Haut Direkt Nachweisbar? *Med. Klin.* 22: 851, 1926.
8. Miyazaki, K.: Ueber die Resorption der Haut, *Jap. J. Dermat. & Urol.* 31: 637, 1931. Abstract in German language, *Jap. J. Dermat. & Urol.* 31: 40, 1931.
9. Neale, R. C.: Personal communication.
10. Neuwirth, E.: Treatment of Rheumatoid Arthritis With Mud, *Arch. Phys. Therapy* 18: 338, 1937.
11. Vaughan, S. L.: The Examination of Urine for Indol, *Proc. Soc. Exper. Biol. & Med.* 29: 623, 1932.
12. Vaughan, S. L.: The Formation of Indol in Urine, *J. LAB. & CLIN. MED.* 22: 399, 1937.

135 WEST 79TH STREET

THE IODINE CONTENT OF BLOOD, URINE, AND SALIVA OF NORMAL PERSONS IN THE NEW YORK CITY AREA*

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SINCE the numerous studies on the iodine content of body fluids have been adequately reviewed in the recent books by Elmer,¹ McClendon² and Salter,³ only the pertinent literature will be referred to in this report.

MATERIAL AND METHODS

Blood, urine, and saliva were obtained from 22 normal persons of whom 9 were males and 13 were females. Their ages ranged from 13 to 51 years. The usual hospital personnel (physicians, nurses, technicians, etc.) served as a major source of material but those assigned to surgery were uniformly excluded because of probable contact with iodine. No attempt was made to standardize the antecedent diet. All materials used for the collection of specimens, such as syringes, needles, and containers, after thorough cleansing, were rinsed with iodine-free distilled water. Iodine-free alcohol was used to sterilize the skin prior to venipuncture.

Urine collections were made over a twenty-four-hour period; blood and saliva were obtained usually after breakfast, and approximately twelve hours after the collection of the urine was begun. The method of Trevorrow and Fashena⁴ was used throughout.

RESULTS

The results are given in Table I. The total blood iodine ranged from 3.1 to 8.4 micrograms per 100 ml. of whole blood, with a mean of 5.9 ± 1.3 . The concentration of iodine in the urine varied from 6.2 to 56.7 micrograms per 100 ml., with a mean of 21.1 ± 16.7 . The total output of iodine in the urine for twenty-four hours ranged from 18.0 to 483.1 micrograms, with a mean of 190.8 ± 138.0 . The salivary iodine varied from 3.5 to 24.0 micrograms per 100 ml., with a mean of 10.2 ± 5.1 .

DISCUSSION

It is generally appreciated that the normal range for blood iodine reported by some of the earlier investigators is undoubtedly too high. Riggs and Man⁵ have recently analyzed critically the various procedures and have pointed out the discrepancies inherent in the older methods of chemical analyses. The comparatively high normal values recorded in the literature may further be ascribed to the selection of persons who inadvertently ingested iodine or who were ex-

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posed to it in some form. Greene and one of us (M. B.)⁶ have stressed this point. In the present series, the average concentration of 5.9 ± 1.3 micrograms of iodine per 100 ml. of whole blood agrees closely with the findings of some recent investigators⁷ and is slightly higher than that recorded by another group.⁸

Baumann and Metzger^{8c} maintained that the blood iodine of males was higher than that of females, an observation which was not confirmed by Fashena,^{7b} Turner and co-workers,^{7c} and Riggs and Man.⁵ The present studies are in agreement with the latter group. The average blood iodine in the 9 males was 5.7 micrograms per 100 ml. and in the 13 females, 6.0 micrograms per 100 ml.

TABLE I

THE IODINE CONTENT OF BLOOD, URINE, AND SALIVA OF NORMAL PERSONS

SUBJECT NO.	AGE	SEX	BLOOD IODINE ($\mu\text{g}/100 \text{ ml.}$)	URINE			SALIVARY IODINE ($\mu\text{g}/100 \text{ ml.}$)
				VOLUME ($\text{ml.}/24 \text{ hr.}$)	IODINE ($\mu\text{g}/100 \text{ ml.}$) ($\mu\text{g}/24 \text{ hr.}$)		
1	40	M	4.4	1,880	19.1	359.1	9.3
2	26	M	7.0	1,200	27.7	332.4	9.8
3	28	F	6.0	2,640	10.2	269.3	20.4
4	38	F	6.9	640	54.6	349.4	13.4
5	24	F	6.8	630	6.2	39.1	10.5
6	23	F	8.4	560	7.5	42.0	5.5
7	26	M	3.1	273	6.6	18.0	6.7
8	22	F	6.7	490	11.5	56.4	7.8
9	42	F	5.7	530	21.9	110.1	6.7
10	24	F	4.9	668	19.2	128.3	10.9
11	13	M	5.7	623	7.0	43.7	7.0
12	51	F	7.0	810	12.6	102.1	24.0
13	14	F	5.4	695	11.8	82.0	
14	25	M	6.9	715	55.8	399.0	15.7
15	29	M	6.5	1,695	14.8	250.9	3.8
16	25	F	3.6	1,071	10.3	110.3	3.5
17	25	F	5.6	761	14.7	111.9	6.1
18	32	M	5.5	852	56.7	483.1	6.6
19	17	F	3.8	1,152			11.1
20	36	M	6.5	990	36.1	357.4	15.0
21	28	F	7.5	765	30.6	234.1	14.7
22	30	M	5.4	1,450	8.4	121.8	4.5
Range			3.1-8.4		6.2-56.7	18.0-483.1	3.5-24.0
Mean			5.9		21.1	190.8	10.2
Standard deviation			± 1.3		± 16.7	± 128.0	± 5.1

The excretion of iodine in the urine of normal individuals varies directly with the intake of iodine in food and water. The urinary loss of iodine, therefore, is dependent upon the geographic location. In goitrous regions the excretion of iodine in the urine is approximately 50 micrograms or less in twenty-four hours.⁹ In the New York City area, where food and water are iodine rich, the present study shows a wide range in the excretion of iodine. The average iodine loss in the urine in the 22 normal persons was 190.8 ± 138.0 micrograms in twenty-four hours.

In contrast to the large number of studies recorded in the literature on blood and urinary iodine, few investigations have been carried out on the concentration of iodine in the saliva.¹⁰ Schneider and Widmann^{10b} reported that the salivary iodine may vary from 0 to 362 micrograms per 100 ml., but this range is altogether too wide. In view of the results obtained in the present

studies, some of their subjects were probably ingesting abnormal amounts of iodine. The range observed in the 22 normal persons in this series was 3.5 to 24.0 micrograms per 100 ml. of saliva, with an average of 10.2 ± 5.1 .

CONCLUSIONS

1. The concentration of iodine in whole blood of 22 normal persons in the New York City area varied from 3.1 to 8.4 micrograms per 100 ml., with a mean of 5.9 ± 1.3 . In 9 males the mean was 5.7 micrograms per 100 ml. and in 13 females, 6.0 micrograms per 100 ml.

2. The concentration of iodine in the urine varied from 6.2 to 56.7 micrograms per 100 ml., with a mean of 21.1 ± 16.7 . The total output of iodine in the urine in twenty-four hours varied from 18.0 to 483.1 micrograms, with a mean of 190.8 ± 138.0 .

3. The concentration of iodine in the saliva varied from 3.5 to 24.0 micrograms per 100 ml., with a mean of 10.2 ± 5.1 .

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REFERENCES

1. Elmer, A. W.: Iodine Metabolism and Thyroid Function, London, 1938, Oxford University Press.
2. McClendon, J. A.: Iodine and the Incidence of Goiter, Minneapolis, 1939, Univ. Minnesota Press.
3. Salter, W. T.: The Endocrine Function of Iodine, Cambridge, 1940, Harvard University Press.
4. Trevorrow, V., and Fashena, G. J.: The Determination of Iodine in Biological Material, *J. Biol. Chem.* 110: 29, 1935.
Fashena, G. J., and Trevorrow, V.: A Note on the Determination of Iodine in Biological Material, *J. Biol. Chem.* 114: 351, 1936.
5. Riggs, D. S., and Man, E. B.: A Permanganate Acid Ashing Micromethod for Iodine Determinations. I. Values in Blood of Normal Subjects, *J. Biol. Chem.* 134: 193, 1940.
6. Greene, C. H., and Bruger, M.: The Iodine Content of the Blood in Diseases of the Liver and Biliary Tract: Clinical and Experimental Studies, *J. Clin. Investigation* 18: 481, 1939.
7. (a) Perkin, H. J., Lahey, F. H., and Cattell, R. B.: Blood Iodine Studies in Relation to Thyroid Disease. Basic Concept of the Relation of Iodine to the Thyroid Gland; An Iodine Tolerance Test, *New England J. Med.* 214: 45, 1936.
(b) Fashena, G. J.: A Study of the Blood Iodine in Childhood, *J. Clin. Investigation* 17: 179, 1938.
(c) Turner, K. B., DeLamater, A., and Province, W. D.: Observations on the Blood Iodine. I. The Blood Iodine in Health, in Thyroid and Cardiorenal Disease, and in Leukemia, *J. Clin. Investigation* 19: 515, 1940.
(d) Perkin, H. J., and Lahey, F. H.: The Level of Iodine in the Blood, *Arch. Int. Med.* 65: 882, 1940.
8. (a) Fowweather, F. S.: The Iodine Content of the Blood of the Patients Suffering From Cancer, *Brit. J. Exper. Path.* 11: 400, 1930.
(b) Stevens, C. D.: Determination of Iodine in Biological Materials, *J. LAB. & CLIN. MED.* 22: 1074, 1937.
(c) Baumann, E. J., and Metzger, N.: On the Amount of Iodine in Blood, *J. Biol. Chem.* 121: 231, 1937.
(d) Grauer, R. C., and Saier, E. A.: A Comparison of the Distilling and Dry Ashing Methods for the Determination of Blood Iodine, *Endocrinology* 24: 553, 1939.
(e) Riggs, D. S., and Man, E. B.: loc. cit., reference 5.
9. Curtis, G. M., Puppel, I. D., Cole, V. V., and Mathews, N. L.: The Normal Urinary Iodine of Man, *J. LAB. & CLIN. MED.* 22: 1014, 1937.
10. (a) Elmer, A. W.: loc. cit., reference 1, p. 111.
(b) Lipschitz, W.: Differenzierung des Thyroxinjods vom Anorganischen jod durch die Membranen des lebenden Organismus, *Klin. Wchnschr.* 9: 642, 1930.
(c) Schneider, E., and Widmann, E.: Klinische und experimentelle Untersuchungen zum Problem des Kropfes und der Basedowschen Krankheit. I. Mitt. Untersuchungen zur Frage des Blutjodspiegels, *Deutsche Ztschr. f. Chir.* 231: 305, 1931.
(c) Scheffer, L.: Über Jodbilanz normaler Menschen, *Biochem. Ztschr.* 259: 11, 1933.

LABORATORY METHODS

GENERAL

MECHANICALLY RECORDING BLOOD SEDIMENTATION CURVES*

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ALTHOUGH credit for discovery of the blood sedimentation test is given to the ancients, the test has been in vogue only since it was accidentally re-discovered in 1918 by the Swedish gynecologist, Robin Fahraeus, in his search for a blood test for pregnancy. Since then no less than forty different techniques have been suggested to perform this very simple test. A standard method would certainly enhance its clinical value.

During recent years efforts have been made by several investigators to record mechanically the blood sedimentation phenomenon. Up to the present three such methods have been devised. These three methods are fundamentally alike in that they are based on the principle that light, which will penetrate plasma, is obstructed by the red blood cell column. The first method devised was the silhouette method. Litten, of Germany,¹ in 1929 exposed a tube of sedimenting blood to a light source. The tube was placed in a slot in front of a kymographic drum, which carried light sensitive paper upon which the increasing column of plasma was recorded as the exposed area of the paper, the lower border of the exposed area revealing the blood sedimentation curve. Sulkowitch² devised a very similar instrument in 1934. In 1938 a second method was presented by Lee,³ who published an article describing the camera method for recording blood sedimentation curves. His device makes use of a lens situated between the blood tube, which is placed in a slot in a light-tight box, and a moving photographic plate which records the image of the illuminated plasma. Nichols⁴ reported a similar device with an added improvement of a thermostatic compartment to maintain the blood at normal body temperature. A third method, which as yet has not been published, makes use of a photoelectric cell, exposed to light coming through the blood plasma. The instrument is so designed as to cause the photoelectric cell to move downward with the lower level of blood plasma. This movement in turn operates a pen which records the sedimentation curve as an inked line on paper carried by a kymographic drum.

I have made use of all three of these methods and, in my hands, the simple silhouette method, combined with a thermostatically controlled compartment to maintain the blood at a normal body temperature, proves most satisfactory. The camera method has the disadvantage of making use of an optical system, which

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may make for a source of error not easily detectable. The photoelectric cell method has the distinct advantage of requiring no darkroom technique, inasmuch as the curve is written on paper with an ink tracing, but with this instrument one cannot be sure that the tracing is correct unless one knows that the line of demarcation between plasma and blood cells is definite. With either of the photographic methods, a hazy line of junction will show up as a hazy line, whereas the photoelectric cell method will show up as a sharp line of demarcation regardless of whether or not the zone of junction is clear.

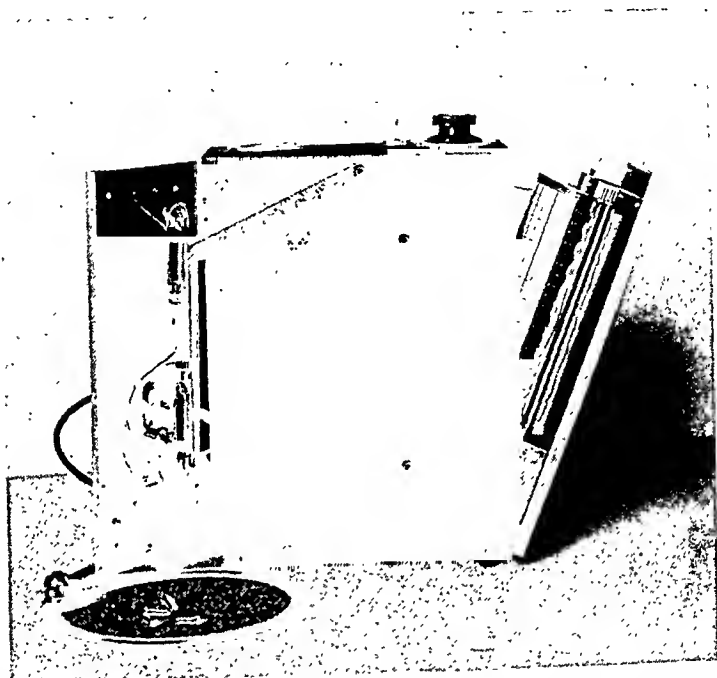


Fig. 1.—Side view of a sedograph with tube in place. The light source compartment has been opened to show the neon tube and transformer. The recording chamber at the right is partly opened, showing the drum.

It has been the common practice of those working with mechanical methods of recording blood sedimentation curves to use the one-hour techniques, especially the Cutler method, probably because it is a well-standardized procedure. I feel that in this point they have been in error. Although the sixty-minute recordings are good methods of ascertaining sedimentation rates when readings are recorded by a laboratory worker, time consumed being a factor, if a machine is to do the test one may just as well see the entire curve without loss of time, and not only a part of the curve as is revealed by the one-hour technique, especially for the bloods of slower sedimentation rates. For instance, two different blood samples which manifest a sedimentation of 5 mm. at the end of one hour in a Cutler tube will also fall 5 mm. in our tube that requires a 30 cm. blood column, because with this slight degree of sedimentation the packing stage influence is not felt. But after the first hour the course of the sedimentation curves of the two bloods may be very different, one sample sedimenting slowly, and the other rapidly. The presedimentation stage is not influenced by the length of the tube. Note in the second sedograph (Fig. 2) that the presedimenta-

tion period requires the entire first hour. Beyond that time the fall may take a slow or a rapid course that can only be determined by reading over a period of time longer than one hour. The longer the tube and time of observation, the more tedious the test but the more accurate the results. Blood samples reveal-

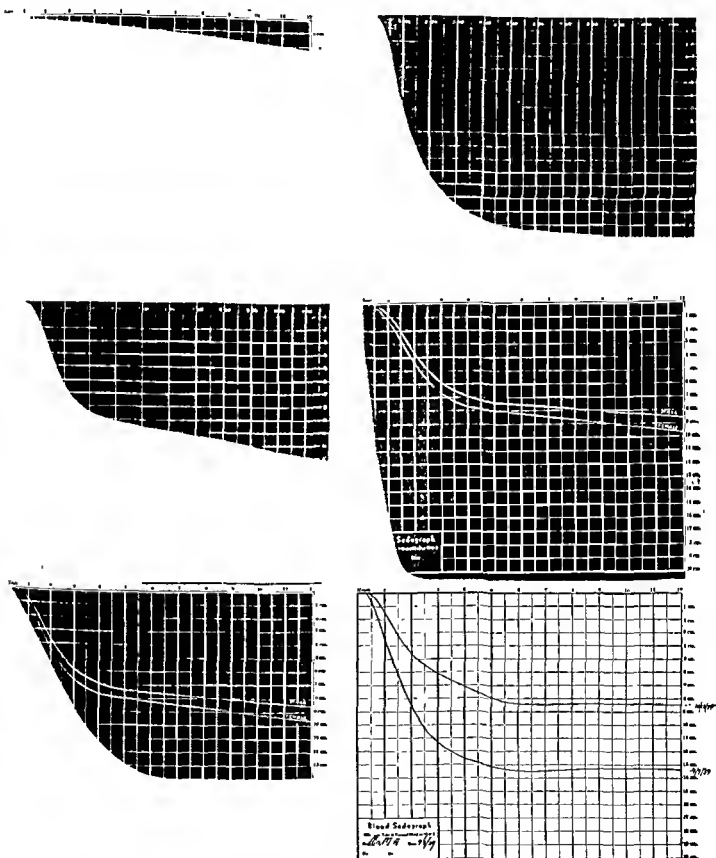


Fig. 2.—Five sedograph records showing different types and degrees of sedimentation curves. In the right lower corner is a piece of tracing paper upon which have been traced two curves from the same individual.

ing identical curves using the one-hour Cutler method have produced unlike sedograph records, one well within the limits of the normal, and the other definitely pathologic.

The silhouette method instrument for recording blood sedimentation at normal body temperature is a device to which I have given the name "sedo-

graph." It is a metal box 5 by 11 by 14 inches, and is divided into three vertical, parallel compartments. In one end compartment is a mercury vapor tube that acts as a light source; in the other end compartment is a slowly revolving drum that carries light sensitive paper which is held in place on the drum by a transparent film mask. On the mask are parallel, horizontal, and vertical black lines, 1 cm. apart, registering as white calibrations the hours of exposure and the centimeters of blood plasma on the final image. The middle compartment contains the blood tube. This compartment is equipped with a heating unit and a thermostatic control which maintains the compartment and the blood tube at

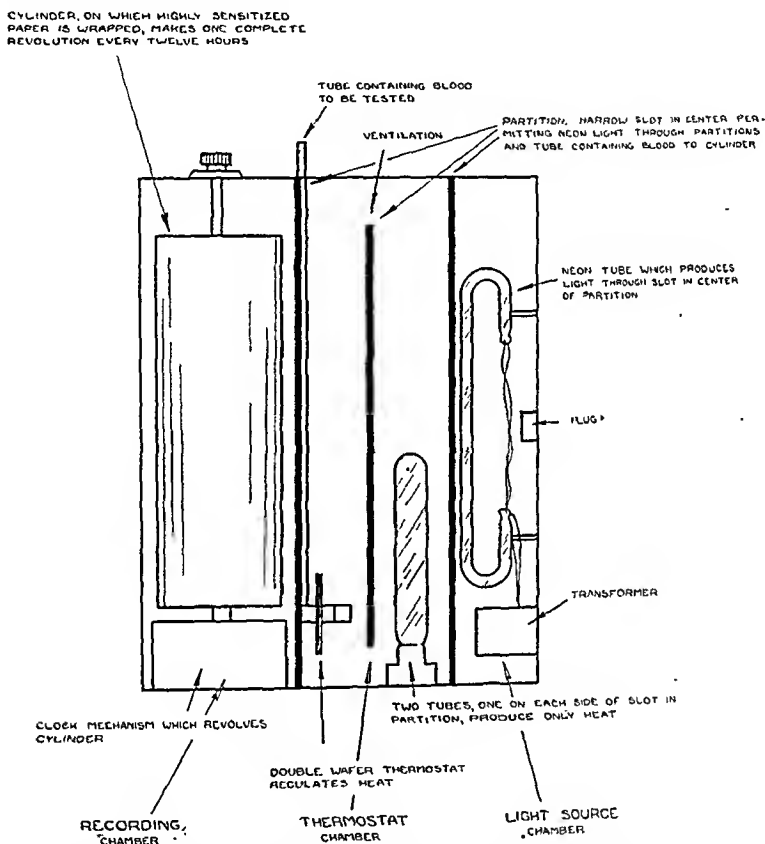


Fig. 3.—Lateral diagrammatic drawing of the sedograph instrument, showing the recording chamber on the left, the light source chamber on the right, and the blood-containing thermostatic-controlled chamber between the two.

a temperature of 99.6° F., thus imitating in vivo conditions. Contrary to general opinion, room temperature is a variable which must be taken into consideration if we are desirous of obtaining exact information from blood sedimentation. Room temperature may vary as much as 30° F. in certain parts of the country, which is too great a change to be disregarded if an accurate record is desired. In some bloods I have found sedimentation rates at 100° F. to be as much as doubled what they are at 70° F. To maintain constant temperature, we have found it simpler to use a heating system that will raise the blood above the outer limits of room temperature, rather than to install a cooling system.

The temperature in the thermostatic compartment is controlled by a wafer thermostat and two 25-watt electric light bulbs which cycle on and off, maintaining constant temperature. This type of thermostational arrangement is used because I have found it to be the one that gives the least lag and the least overshoot at the onset of the test. There is a time interval of about six to ten minutes, depending upon the temperature of the room, during which the thermostational compartment is warming up to blood temperature. Inasmuch as this is the presedimentation period, I feel that it is unnecessary to warm the compartment by inserting an empty tube into the apparatus just prior to the test.

The records make use of $8\frac{1}{2}$ by 11 inch photographic paper, since this is the size paper most commonly used in hospital charts. This produces an accurate, permanent, actual size image of the sedimentation progress, with more detailed information than do smaller images. This instrument uses ordinary double weight glossy photographic paper, Azo No. 3.

A column of citrated blood 300 mm. in length is used, inasmuch as this is the greatest column of citrated blood that can be accommodated by $8\frac{1}{2}$ by 11 inch paper in those cases with increased sedimentation rates. The length of the tube is a guard against deviation from a strictly upright position, the importance of which has been pointed out by numerous investigators. It is more difficult to be sure that the shorter tubes are perpendicular. We use a tube of 4 mm. inside diameter; tubes of larger diameter transmit the required amount of light with difficulty. This is a convenient sized tube to handle and clean.

The test is run for a period of twelve hours, since sedimentation in a tube of this size is complete for most pathologic bloods by that time, whereas the nearer normal pathologic bloods are seldom complete, and the normal blood samples are never completed in a shorter period of time. I feel that it is very desirable to secure the entire sedimentation curve in order to evaluate properly the results of the test.

I have used 3.8 per cent aqueous sodium citrate as an anticoagulant because it is isotonic with the blood and gives a clearer plasma than heparin or the oxalates, which in the case of my technique is a distinct advantage.

A blue light source is used because, with the pale yellow media of the blood plasma and the opaque red cell volume, a more contrasting image is assured.

The technique of the test is as follows:

Four cubic centimeters of blood secured by venepuncture are mixed with 1 c.c. of 3.8 per cent aqueous sodium citrate in an ordinary test tube. The blood is pipetted to the black mark encircling the 4 mm. diameter sedimentation tube, which gives a column of blood 30 cm. long. A cork is placed in the bottom of the tube, and the tube is inserted into the machine. As the tube is placed in the machine, a switch is automatically closed; this starts the synchronous motor that rotates the drum, turns on the mercury vapor tube, and sets into operation the thermostatically controlled compartment. The test from then on is entirely automatic, including an automatic stop after twelve hours, until such convenient time after twelve hours that the operator may choose to remove the tube, take the machine into the darkroom, develop the record, and reload the machine to await the next test.

A simple and easy method of loading and unloading has been devised, and although our mechanism requires a darkroom for these two operations, which

are done in one sitting, I feel that inasmuch as the instrument is not heavy or bulky, the method is technically simpler than if photographic plates were used, because in either case a trip to the darkroom is necessary. The recording chamber is opened by lifting the dial knob, allowing the recording chamber end, which is hinged at the bottom, to swing open. The film mask is opened by disengaging the rubber bordered ends from their hooks, and the photographic paper is slipped under the mask. The chamber door is closed, and the dial is set at the arrow or "start line." The instrument is now ready to be used again.

The entire instrument consumes about 50 watts of electrical power. The synchronous electrical motor operating the drum makes one revolution in fifteen hours. The greatly reduced speed of the electrical motor makes it powerful and dependable. Twelve hours are used for the operating time, the space of the three remaining hours (6 cm.) being used for attachments of the mask and margins of the paper. The drum is 30 cm. in circumference; therefore, a point on the circumference of the drum moves 2 cm. each hour.

The size of the sedograph record, besides permitting attachment to hospital charts, lends itself well to being traced. A sheet of semitransparent tracing paper may be laid over the sedograph record and the curve traced thereon. Serial sedograph curves of a patient may be traced on this same sheet. It becomes a simple matter then for the physician to see at a glance a mechanically made, complete, and accurate sedimentation curve, as well as the most minute differences in serial readings.

I believe that the future will reveal the necessity of this degree of accuracy.

REFERENCES

1. Litten, L.: Photographische Darstellung der Blutkörperchensenkung, München, med. Wehnsehr. 74: 2133, 1927.
2. Sulkowitch, H. W.: Photographic Suspension Stability (Sedimentation Rate) Apparatus, Preliminary Report, Am. J. M. Sc. 187: 65, 1934.
3. Lee, Terence: Sedimentometer: Photographic Recorder of Suspension Stability of Erythrocyte, Am. J. M. Sc. 195: 729, 1938.
4. Nichols, R. E.: New Instrument for Automatically Recording Erythrocyte Sedimentation Rate and Volume Percentage of Cells and Plasma Upon Single Permanent Record, J. LAB. & CLIN. MED. 24: 631, 1939.

A SEMISOLID DOUBLE SUGAR MEDIUM FOR THE IDENTIFICATION OF THE COLON-TYPHOID GROUP*

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RUSSELL'S double sugar medium has been used extensively in bacteriologic practice. By the use of this single medium, it is possible to obtain preliminary information of practical value in laboratory diagnosis. But its general application is limited, owing to the lack of differentiation between the motile *Eberthella typhi* and the nonmotile *Shigella* group, as well as between the motile *Salmonella* and the nonmotile para-colon group of organisms. Moreover, one of us has shown (P. Y. L.)¹ that it is unnecessary to employ both lactose and dextrose in both the slant and the butt, because the purpose of differentiation can be satisfactorily accomplished by using dextrose alone in the butt and lactose alone in the slant.

Since 1938 we have employed a double sugar medium with a semisolid butt to indicate motility. Acid fuchsin has been used as an indicator since Cummings² has shown that this will give satisfactory results. Over 12,000 specimens of stool alone were examined in the past two years by this medium for preliminary identification. A total of over 2,000 positive cultures of various pathogenic gram-negative intestinal organisms was obtained. The following is the formula of this semisolid medium:

Solution A—Sugar-free broth (blood digest broth ³), pH 7.4	100
Agar	0.35
Solution B—Sugar-free broth, pH 7.4	100
Agar	0.9
Lactose	1.0

Sterilize both solutions A and B by autoclave at 8 pounds pressure for fifteen minutes and cool to about 60° C.; add to both solutions A and B 0.5 c.c. of 2 per cent aqueous solution of acid fuchsin.

Keep solution B in water bath at 45° to 50° C. until used.

To solution A add 2 c.c. of a sterile 10 per cent solution of dextrose.

Fill tubes of 12 mm. inside diameter with 2 c.c. of solution A and cool in upright position until it solidifies. Then add to the same tubes solution B also in 2 c.c. amounts and let it solidify in a slanting position. The medium is now ready for use.

ACTUAL PROCEDURE AND INTERPRETATION

The stool specimens are, as usual, plated on the China blue rosolie acid medium, which in our hands have given satisfactory results. Next morning, after

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sixteen to twenty hours' incubation, each of several suspicious colonies picked from every plate is inoculated into a tube of this semisolid double sugar medium. After another twenty-four hours' incubation the results in this medium are read and interpreted according to the scheme in Table I.

TABLE I

RESULTS IN SEMISOLID MEDIUM				TENTATIVE DIAGNOSIS
Slant	Fermentation in Butt	Gas	Production of Motility	
O	A	-	-	<i>Shigella</i>
O	A	-	+	<i>Eberthella</i>
O	A	+	+	<i>Salmonella</i>
O	A	+	-	Para-colon group
A	A	+	+ or -	Colon-aerogenous group
O	O	-	+	<i>Alkaligenes</i>

O = No change. - = Nonmotile or no gas production.

A = Red-color. + = Motile or positive for gas.

For those cultures that show the first three types of reaction, further transfers into sugar fermentation tubes and agglutination with specific serum are made. Cultures showing the latter three types of reaction were discarded and considered to be those of nonpathogenic organisms. Care should, however, be exercised in cultures giving the type of reaction of the para-colon group, since the nonmotile *Salmonella* would give the same reaction. In such cases the amount of acid produced may give some indication, since the organism of the para-colon group usually produces a large amount of gas. If the amount of acid produced is slight, further studies in special sugars are indicated.

COMMENTS

The medium herewith reported makes use of the original principle of Russell's medium but has the following additional advantages:

- (1) The consistency of the butt is sufficiently hard to stand up under the slant and at the same time is suitable for the demonstration of motility.

The use of an inexpensive and stable indicator—acid fuchsin.

The elimination of one of the sugars from both butt and slant, found unnecessary, effects material economy when large numbers of stool cultures have to be examined daily.

- (4) Blood digest medium has been found to be sugar free and to support growth for the organisms examined without the addition of peptone.

REFERENCES

1. Liu, P. Y., and Li, S.: Further Studies on Russell's Medium, Chinese M. 247, 1938.
2. Cummings, G. D.: The Use of Acid Fuchsin in Triple Sugar Medium. Dis. 47: 359, 1930.
3. Stickel, J. E., and Meyer, K. F.: Peptic and Triple Sugar Mediums for Routine Bacteriologic Culture Media. Infect. Dis. 1: 1, 1930.

DECREASED FRAGILITY OF ERYTHROCYTES INTERFERING WITH LEUCOCYTE COUNT*

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INCREASED resistance of erythrocytes to hypotonic solutions occurs infrequently. It is known to be a transitory phenomenon in hypochromic anemias, particularly after the loss of a large amount of blood, in some cases of obstructive jaundice and in pneumonia. The discovery of such decreased fragility requires the special technique of the commonly used fragility test. Whether cells more resistant than usual to hypotonic solutions are more resistant to other hemolytic agents has not been definitely determined. The following case is reported to illustrate the possibility of increased resistance of erythrocytes to acetic acid to the point where it interfered with the routine technique for counting leucocytes. The circumstances surrounding the discovery of this unusual phenomenon are as follows:

The patient was a 31-year-old woman, gravida iv, who had a breech extraction of a premature dead fetus (twenty-six weeks' gestation) on August 10. The placenta separated slowly and with difficulty, and there was considerable loss of blood. Her red blood cell count dropped in two days to 2,180,000, while she continued to pass blood clots from the vagina. In spite of transfusions on August 12 and 13, the erythrocyte count dropped to 1,820,000 on August 14. The color index varied between 1.01 and 1.05. Leucocyte counts were performed on August 12 and 13 (20,600 and 12,600), and nothing unusual was noted while doing these tests.

On August 14 the laboratory technician reported that she was unable to do a leucocyte count because of a large amount of what looked like debris in the counting chamber. Thinking she might have used a solution with precipitate of some kind in it or a dirty pipette, she attempted the count a second time with freshly made solutions; she obtained the same result. A third attempt still showed debris. In the meantime the solution had been used without difficulty on many other patients. The next morning, August 15, she was still unable to count the cells. At this time, examination of the debris suggested that it might be made up of clumped and distorted erythrocytes, and several rough tests were performed to test this hypothesis.

Blood was drawn from the vein, and enough sodium citrate was added to prevent clotting. A drop of this blood when added to the 1 per cent acetic acid usually used for leucocyte counts gave a rather muddy precipitate, and microscopic examination of this precipitate showed clumped red blood cells which could, however, be separated by pressure on the cover slip. A drop of blood was placed in 2 c.c. of 0.85 per cent sodium chloride solution. The red blood cells, for the most part, appeared perfectly normal microscopically, but a few of them were bowl shaped. A drop of blood was placed in 2 c.c. of distilled water. Many cells, of course, hemolyzed; but some cells did not hemolyze even after about fifteen minutes, and examination of these unhemolyzed cells showed nearly every one to be bowl shaped instead of the usual biconcave disc. Since this type of cell has been described¹ and shown to have decreased fragility or increased resistance to hypotonic solutions, it seemed possible that the cells in this case might also be abnormally resistant to acetic acid. Accordingly, attempts were made

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to count leucocytes using stronger solutions instead of 1 per cent acetic acid. With 2 per cent acetic acid a small amount of debris was still noted, enough to interfere with a reliable leucocyte count; with 3 per cent acetic acid used as diluting fluid, the field was clear and a leucocyte count could be made without difficulty. During the remainder of the patient's stay in the hospital white blood cell counts were made daily, using both 1 per cent acetic acid and 3 per cent acid as diluting fluids. On August 16, 3 per cent acid was required, but on August 17 and on succeeding days, it was possible to make the leucocyte count with the usual 1 per cent acid.

In the meantime the patient's erythrocyte count rose slowly, and on August 22 she was discharged from the hospital with a count of 3,200,000. The patient's blood type was type O.

Only a few additional tests were performed in the interval during which the increased resistance to acetic acid was demonstrable. The fragility test was inconclusive, for it was not read until twelve hours after its performance, and it is possible that if it had been read earlier it might have shown more striking results. On August 16 hemolysis was begun at 0.44 per cent sodium chloride and was complete at 0.32 per cent, a result not strikingly different from the normal and one which was not expected after the experiments with distilled water. Before the patient's discharge from the hospital a second test demonstrated hemolysis to begin at 0.46 per cent and to be complete at 0.36 per cent. Hence while the first test was at the lower limit of normal, there was a striking shift simultaneous with the change in resistance to acetic acid.

A galactose tolerance test was normal. The icterus index was 3, and cholesterol in the blood was 205 milligrams per cent.

The fragility of erythrocytes seems to be correlated with the shape of the cell. It is possible to devise a purely mechanical explanation which, at first glance, seems to fit the facts; but there seems to be some doubt as to whether this type of explanation is completely satisfactory. A spherical cell should be able to swell only slightly without disruption, and consequently will hemolyze in slightly hypotonic solutions. The normal biconcave disc can perhaps be conceived of as a partially collapsed cell and should have a greater capacity for taking up fluid than the spherical cell; this, of course, is exactly what happens. A bowl-shaped cell, such as that described by Barrett,¹ should have an even greater capacity for absorbed fluid and, therefore, a greater resistance to hypotonic solutions. The experiments of Barrett seem to show that these cells are resistant to 0.30 per cent sodium chloride and even lower concentrations, and my rough experiments with distilled water in this case seem to bear out this supposition.

The resistance of the cell to acetic acid, however, cannot be explained easily on this purely mechanical hypothesis and suggests that while the shape of the cell is definitely of importance, the physical and/or chemical structure of the cell also varies and is of importance. It is desirable that we know whether resistances to acid and to hypotonic solutions are parallel. The older work seems to indicate that resistance to hypotonic solution and to other hemolytic agents are parallel,² but Dameshek and his co-workers³ suggest that there may be "differential fragilities." Mason and Rockwood⁴ found fragility to any hemolytic agent to vary with the hydrogen-ion concentration. Furthermore, the possibility of the reversal of hemolysis,⁵ as well as other experiments, suggests that rupture of a cell is not even a necessary part of hemolysis.

The bowl-shaped cell described assumes a peculiar form in dried smears, known as the target cell.¹ This is due to a characteristic indentation which takes

place during the drying of the cell, so that there is a central zone of hemoglobin-containing material, a midzone of little or no hemoglobin, and a peripheral zone again of hemoglobin. In our case, about 1 per cent of the cells in dried stained smears show this characteristic target appearance.

Inquiry among our technicians disclosed two previous instances in our laboratory in which it was not possible to perform the leucocyte count because of "debris." In both cases the phenomenon was transitory, lasting in one case one day, in the other, two days; but nothing was done to trace the cause of the phenomenon in these previous cases. The transitoriness of the increased resistance to acetic acid suggests that it may often be missed when repeated leucocyte counts are not performed.

SUMMARY

A case is reported in which, for a period of three days, it was not possible to perform a leucocyte count using the usual 1 per cent acetic acid as diluting fluid. It was, however, possible to make this count if 3 per cent acetic acid was used.

The cause for the difficulty in performing the leucocyte count was the marked increased resistance of the erythrocytes to acetic acid. The erythrocytes were also hyperresistant to hypotonic salt solution, and for a short time some of them were not hemolyzed even by distilled water.

The hyperresistant cells were bowl shaped in wet smears. Probably corresponding to these, target cells were noted in dry smears.

REFERENCES

1. Barrett, A. M.: A Special Form of Erythrocyte Possessing Increased Resistance to Hypotonic Saline, *J. Path. & Bact.* 46: 603, 1938.
2. Pierce, R. M., Krumbhaar, E. B., and Frazee, C. H.: *The Spleen and Anemia*, Philadelphia, 1918, J. B. Lippincott Co., Section I.
3. Dameshek, W., and Schwartz, S. O.: Acute Hemolytic Anemia, *Medicine* 19: 231, 1940.
4. Mason, E. C., and Rockwood, Reed: Some Physicochemical Aspects of Hemolysis, *J. LAB. & CLIN. MED.* 10: 10, 1925.
5. Lit. in Isaacs, R.: *The Erythrocyte*, Downey's Handbook of Hematology, New York, 1938, Paul B. Hoeber, Vol. 1, pp. 106-112.

AN AID IN THE INJECTION OF THE EAR VEIN OF THE RABBIT

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VARIOUS means have been advocated for facilitating the injection of solutions into the ear veins of the rabbit, such as removing the hair, placing a paper clip on the ear, rubbing with xylol, and holding the rabbit in a box. All these procedures are of some help, but they leave much to be desired in certain cases.

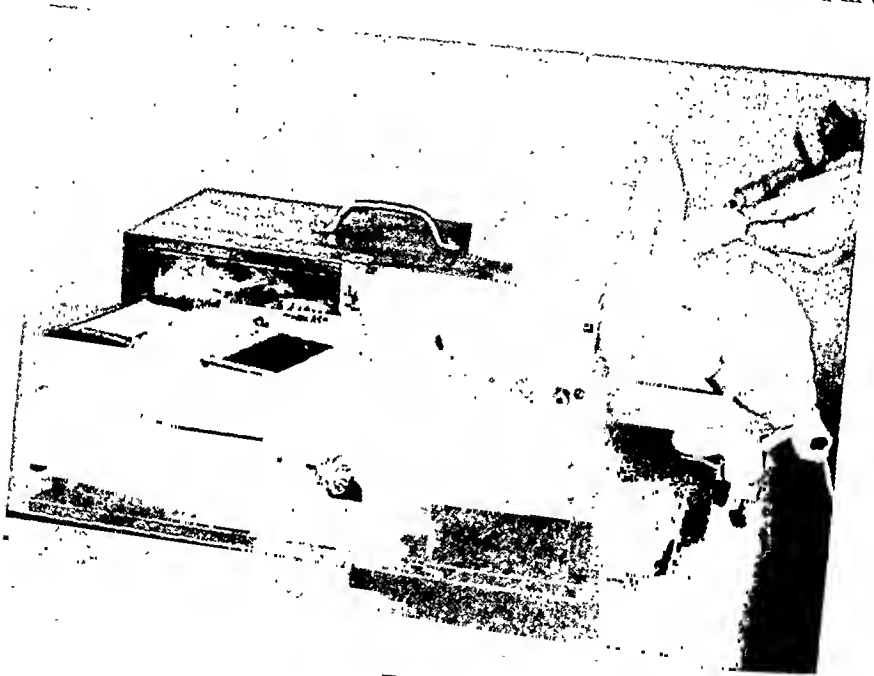


FIG. 1.

A simple arrangement that we have used in this laboratory for over three years, and that has proved very satisfactory, consists essentially in the use of a light placed below the rabbit's ear. All the veins stand out in view, so that one can be sure the needle is in, and by watching the blood being pushed back by the injected fluid can be certain that the solution all goes in the vein and not out into the tissues.

A light unit attached to the rabbit box makes a compact assemblage ready for use at any time. However, a spotlight bed lamp, which can be obtained at almost any hardware or department store, works very well. It can be clamped to the edge of the table so that the rabbit's ear can be held out over the lens. Fig. 1 shows this type of lamp in use, together with the light unit referred to above detached from the box.

*From the Laboratory of the Myers Clinic, Philippi.
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Some rabbits will remain quiet with little or no restraining and it is an easy matter to get into a vein and to stay in, but it is more particularly for those many other rabbits that are not so docile that this method is most useful. Also, where oft-repeated injections are done, the veins can be kept in better condition by the more easy and accurate venepunctures; furthermore, use is permitted of more and smaller veins.

When a rabbit moves his ears so vigorously that it is difficult to keep the needle in the vein, the needle can usually be kept in position by holding it and the ear of the rabbit together by pressure with the thumb on the hub of the needle against the fingers below the ear, and at the same time pulling slightly on the ear.

COMPOSITION TUBES AS A SUBSTITUTE FOR GLASS IN THE ISOLATION OF SPORULATING ANAEROBES

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EXPERIMENTS have been conducted for some time with the cultivation of sporulating anaerobes in tubes constructed of substances other than glass. Experiments thus far conducted seem to warrant a brief report, together with continued investigation.

The early experiments were conducted with small, spirally wound cellulose tubes, sold widely under the name of "Glassips." These were plugged with cotton at the upper end and sterilized in large glass tubes by baking at 160° C. for three hours. No extensive alteration of the tubes was effected by this heating.

Customary culture dilutions were made in melted tubes of suitable anaerobe agar. Each dilution was drawn up into separate pipettes ("Glassips") to a height of about 10 cm. The lower tip of each pipette was then dipped into a dish of sealing wax kept just above the melting point. These pipette cultures were then incubated at 37° C. as usual.

In these tubes we successfully cultivated *Cl. perfringens* and *Cl. tertium*. *Cl. sporogenes*, a much more strict anaerobe, grew only intermittently, and gave many failures. *Cl. botulinum* type A did not grow in any trial.

While this established the principle, it seems that these very thin-walled tubes do not maintain a sufficient degree of anaerobiosis to warrant their use for the purpose desired.

We next experimented with a larger and thicker-walled tube, sold commercially as a container for toothbrushes. This tube is of test tube construction, and is about 15 cm. in length and 2 cm. in diameter. It is composed, presumably, of some form of cellulose. This tube was plugged and baked. Melted whey agar was inoculated with *Cl. beijerinckii* and poured into the composition tube, hardened, then incubated at 37° C. Within twenty-four hours the colonies were visible to the naked eye.

A control culture in a glass tube showed much more vigorous growth and extensive gas formation, while gas did not appear in the composition tube until after forty-eight hours. It was noted also that, in the composition tube, the colonies developed chiefly in the center of the agar column, not approaching closer than about 2 mm. from the wall of the tube. We presume from this either that the material in the wall of this composition tube is not an absolutely effective seal against oxygen, or that possibly some toxic agent may diffuse slightly from the wall into the medium. The former suggestion is the more probable explanation.

However this may be, the plastic tube served effectively to grow, in a deep agar column, an anaerobic organism which consistently offers difficulty in ordinary plate isolation.

The chief advantage of this proposed method of cultivation is that the colonies may be reached directly through the wall without cracking the tube, as is the usual procedure with glass tubes, or by slipping out the agar column, as is the case with the use of the Burri tube. Both of these methods offer opportunity for contamination unless the tube contains only a single species.

The wall of the composition tube may be washed with suitable disinfectant (it cannot be flamed), and a dissecting needle is heated to red heat. It is then easily and instantly inserted through the wall of the tube opposite the desired colony. A Pasteur pipette tip is heated and drawn to a fine capillary. This tip is snipped off and inserted through the hole into the colony. The semifluid colony contents can be observed to enter the capillary, which is then inserted into a tube of suitable sterile culture medium and tapped to break off the tip.

We are now seeking a variety of sizes of tubulatures of similar resin compounds, and hope to report more extensive experiments in the near future.* Horsfall¹ has recently reported the use of celluloid tubes for the preservation of frozen viruses. We believe that similar composition tubes may offer a new and effective means of anaerobic isolation, as applied especially to certain species which do not respond readily to the usual plating methods.

It may be added that this principle has been applied experimentally to plating, using the original Koch mica sheet cover idea, but substituting flat sheets of cellulose. We must admit that, thus far, the results are only imperfectly successful. Only *Cl. perfringens* and *Cl. tertium* have responded. We believe that thicker sheets of cellulose, together with greater depth of agar in the plates, may give more satisfactory results. At least the preliminary results appear to warrant continuation and modification of the experiments.

REFERENCE

1. Horsfall, F. L., Jr.: A Low Temperature Storage Cabinet for Preservation of Viruses, *J. Bact.* 40: 559, 1940.

*Since acceptance of this paper for publication, tubes and open tubing in a variety of plastic materials of suitable sizes and wall thicknesses have been obtained from the Dow Chemical Company, Tennessee Eastman Corporation, and the Lusteroid Container Corporation. The applicability of these to the problem of anaerobic isolation will be reported upon in a subsequent publication.

INEXPENSIVE SPECIFIC GRAVITY APPARATUS*

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DURING the past few years the determination of specific gravity by a more accurate technique, such as the falling-drop method of Barbour and Hamilton,¹ has increased tremendously in popularity. In many cases the specific gravity of the blood gives the essential information that much more time-consuming and costly albumin and globulin determinations convey. The method is simple and can be performed readily in a doctor's office in a few minutes, thus obtaining the results without delay and with very little inconvenience for the patient. In the medical school curriculum the student should be taught how to use this equipment, and the significance of such determinations with regard to blood, serum, urine, and body fluids. The chief drawback has been the expense involved in providing the high-priced manufactured outfits in sufficient numbers as routine physiology and pharmacology laboratory equipment for regular classroom use.

An inexpensive satisfactory specific gravity apparatus was devised, using a regular 2 liter unruled graduate cylinder as the container, for this holds sufficient water so that temperature does not fluctuate excessively. Two circular discs of sheet brass, $\frac{3}{16}$ inch thick, were cut to fit the graduate cylinder, with just enough play so that removal from the container was easy. Four holes were drilled into each disc (Fig. 1) for the glass tubes,[†] with a bore between 7.45 and 7.55 mm., as described by Barbour and Hamilton.¹ A central hole was drilled in each just large enough to allow a brass rod, $\frac{1}{4}$ inch in diameter, to pass through. One brass disc was then soldered flush with the end of the brass rod, and the other disc was soldered on the rod so that the distance was $2\frac{1}{2}$ inches from the top of the cylinder, when the first disc was placed on the bottom inside the container. The four holes in each disc were lined up in proper position at the time of soldering, one above the other, so that the glass tubes were held in a true vertical position. The central brass rod was cut off $1\frac{1}{2}$ inches above the upper disc and used as a handle, and the grip was improved by fitting a round nut on the end. Three legs were made from the brass rod and attached to the lower brass disc by drilling proper size holes as close to the outside edge as possible and soldering them in place. Each leg was made an inch long and spaced equally distant, one from the other. Two small holes were drilled in the upper disc, one on each side, between the holes for the glass tubes, each for a

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†The glass tube should be about 50 cm. long and the bore must be carefully checked at each end and fall within the prescribed dimensions. The tubes are marked with lines that extend all the way around exactly 30 cm. apart, which is the distance used in determining the drop falling time with a stop watch graduated in one-tenth seconds. The tube can be cleaned easier if the lower end is left unsealed and closed with a cork.

long stem centigrade thermometer. A small brass holder with a piece of brass placed through the open ring on the upper end was used to hold the thermometer in place (Fig. 2). Thus the thermometers were placed in a convenient position for reading and for accurate determinations of the temperature. Small brass rings, 1/16 inch wide, were placed around the glass tubes, containing the xylene-brombenzene mixture, exactly 30 cm. apart from inside edges. These



Fig. 1.



Fig. 2.

Fig. 1.—Photograph of stand for holding specific gravity tubes.

Fig. 2.—Photograph of stand in glass graduate containing specific gravity tubes in place.

increased the accuracy of stop watch readings of the drop falling time over plain scratch marks on the glass. All the apparatus placed in the glass graduate (see Fig. 2) can be removed together at once and quickly, which makes cleaning the tubes and refilling easy, or convenient for storing equipment. The rigid support holds the glass tubes firmly and securely in place. There are four tubes containing four different xylene-brombenzene mixtures, thus insuring an adequate range.

A one-handed controlled pipette holder, as described by Guthrie,² has been very satisfactory in this laboratory for use with the special 10 c.mm. pipette for measuring the size of the drop. A good technician can make a perfectly satisfactory holder at a very low cost.

The total cost of parts for this apparatus for the department at Columbia, Mo., was less than \$2.00, exclusive of the labor, stop watch, thermometer, and pipette. The laboratory technician assembled the parts without difficulty. In case of breakage of the glass cylinder, the apparatus could be transferred to another quickly. Using the foregoing method for constructing the apparatus, one such piece of equipment can be supplied each four or five students without excessive cost, and accurate results can be secured for research as well as for teaching purposes. In many instances the specific gravity determinations training would be very valuable, for probably the specific gravity of the blood would be obtained in many cases which otherwise would be neglected if it were necessary to obtain total protein or albumin-globulin determinations.

REFERENCES

1. Barbour, H. G., and Hamilton, W. F.: The Falling Drop Method for Determining Specific Gravity, *J. Biol. Chem.* 69: 625, 1926.
2. Guthrie, C. C.: An Apparatus for Quickly Measuring the Specific Gravity of Body Fluids, *J. LAB. & CLIN. MED.* 17: 1158, 1932.

THE TECHNIQUE FOR THE ISOLATION OF BRUCELLA FROM HUMAN BRUCELLOSIS*

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INTRODUCTION

THE isolation of *Brucella* from human sources offers considerable difficulty. Various methods have been described,¹ all of which must be modified at times, depending upon the source of the material which is being cultured.²⁻⁶ The procedures described here have been developed for routine use in the biologic division of Duke Hospital for the isolation of *Brucella* from blood, bile, lymph nodes, joint fluid, spinal fluid, feces, urine, and necropsy specimens.

METHODS

Blood.—Fifteen cubic centimeters of the patient's blood are introduced into a flask containing 4 c.c. of 2.5 per cent sodium citrate solution. Two cubic centimeters of the citrated blood are added to each of four deep tubes (15 c.c.) of molten liver infusion agar, cooled to 40° C. for pouring plates. Two cubic centimeters of blood are added to each of two flasks containing 100 c.c. of beef infusion broth, pH 7.4. Half of the cultures are incubated in the presence of 10 per cent carbon dioxide.

All cultures are examined daily. After the first forty-eight hours' incubation, if no colonies are visible on the plates, 5 c.c. amounts of the supernatant broth are transferred from the flasks to sterile centrifuge tubes. After centrifugalization at high speed for fifteen minutes, the supernatant fluid is dis-

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carded, a loopful of the sediment is streaked over the surface of a liver infusion blood agar plate, and on a slide for a stained smear. The flasks are then shaken to mix the blood and broth, and 10 c.c. of this mixture are transferred to each of two flasks of fresh broth.

The colonies of *Brucella* in a poured plate containing blood resemble those of *Strep. viridans*, if such colonies are deep in the medium. After further incubation, especially if growing to the surface, the colony appearance resembles more closely that of *E. typhosa*. The colonies are picked and streaked over the surface of a liver infusion blood agar plate. The transplants are incubated under the same conditions as the original cultures. This procedure is repeated at two- to three-day intervals, until sufficient growth is obtained for identification. *Blood cultures for Brucella should be kept for eighteen days before being reported as negative.*

After there is sufficient growth, tubes of dextrose, lactose, maltose, mannite, sucrose, and plain beef infusion broth, pH 7.4, are inoculated. A non-motile gram-negative coccobacillus, which ferments none of the carbohydrates and is agglutinated by antibrucella serum is diagnostic of *Brucella*. The bacteriostatic reaction of dyes⁶ and agglutinin-absorption tests⁷ are used to differentiate the species of *Brucella*.

These cultures may be supplemented by the inoculation of 2 c.c. amounts of the citrated blood into the peritoneum of each of two guinea pigs. *The animals are kept for three months if necessary before they are killed.* The spleen, liver, peritoneal fluid, and lymph nodes (if enlarged) of the animals are cultured for *Brucella*.

Bile From Duodenal Drainage.—The "B" bile is used for culture. One-tenth cubic centimeter of bile is added to each of four tubes containing 10 c.c. of beef infusion broth, pH 7.4, and the same amount of bile is streaked over the surface of each of two liver infusion blood agar plates. Half of the cultures are incubated in the presence of 10 per cent carbon dioxide.

The cultures are examined daily after the first forty-eight hours' incubation. If the broth tubes are cloudy, 5 c.c. amounts are transferred to sterile centrifuge tubes. After centrifugalization at high speed for fifteen minutes, the supernatant fluid is discarded, and a loopful of the sediment is streaked over the surface of a liver infusion blood agar plate. A smear of the sediment is made and stained by Gram's method. If the broth tubes are not cloudy, 1 c.c. is transferred to tubes of fresh broth, and after forty-eight hours' incubation, the procedure just described is repeated.

On the streaked plates after twenty-four to seventy-two hours' incubation, the colonies are barely visible to the naked eye, appearing as minute, transparent colorless drops. *Microscopically, these are made up of small noncharacteristic gram-negative bodies, and one not familiar with the growth habits of Brucella may not identify this organism.* However, after transferring to fresh liver infusion blood agar and incubating for twenty-four to forty-eight hours, the colonies are larger and more numerous, and the organism is more typical in the Gram's stain. The transplants are incubated under the same condition as the original cultures. This procedure is repeated at two- to three-day intervals,

until there is sufficient growth for completing the identification. *The cultures should be kept for fourteen days before they are reported as negative.*

Lymph Nodes.—Lymph nodes removed by biopsy are cut immediately into small pieces with sterile scissors. Pieces of the node are streaked over the surface of two liver infusion blood agar, or North's gelatin blood agar, slants, or plates. One slant or plate is incubated in an atmosphere of 10 per cent carbon dioxide. If slants are used, the upper portion of the tube is heated in the flame to expel the air, which is then replaced by blowing the breath into the tube through a sterile plugged pipette, and inserting immediately a sterile rubber stopper. By this procedure a concentration of approximately 10 per cent carbon dioxide is obtained.

The cultures are examined daily. The first evidences of growth are tiny colonies resembling droplets of moisture, which may appear after forty-eight to seventy-two hours' incubation. *Stained smears from these colonies show minute coccoid bodies with indefinite outlines.* Transplants are made to fresh media every two to three days after the first seventy-two hours' incubation, even though there may be no visible growth. After one or two transplants the colonies are larger and stained smears show the usual coccoid or bacillary forms. The organism is identified in the usual manner. *The cultures are kept for three weeks before they are reported as negative.*

The remaining portion of the lymph node is ground in a sterile mortar, to which are added 2 c.c. of sterile physiologic saline. One cubic centimeter of this mixture is injected into the groin of each of two guinea pigs. The animals are followed in the same manner as those injected with blood.

Joint Fluid.—The fluid should be planted at the bedside, and it is essential that the medium used should be warmed to body temperature before it is used for inoculation. Two-tenths cubic centimeter of fluid is streaked over the surface of each of four liver infusion blood agar slants, and the same amount of fluid is put into each of two tubes containing 10 c.c. of beef infusion broth, pH 7.4. Two of the slants are incubated under increased carbon dioxide tension.

The cultures are examined daily, using the same routine as for lymph node cultures. *Cultures of joint fluid are kept for three weeks before they are reported as negative.*

Spinal Fluid.—One cubic centimeter of a 1:100 dilution of inactivated polyvalent antibrucella rabbit serum is mixed with 5 c.c. of spinal fluid and incubated at 37° C. for thirty minutes. After incubation the spinal fluid serum mixture is centrifuged at high speed for fifteen minutes, and 0.2 c.c. of the sediment is planted on each of four liver infusion blood agar slants, and into each of two tubes of beef infusion blood broth, pH 7.4. Two of the slants and 1 tube of broth are incubated under increased carbon dioxide tension.

The cultures are examined daily. The same routine as for lymph nodes is used for the blood agar slants, and the technique for the bile cultures is used for the broth cultures.

Feces.—About 1 Gm. of feces is mixed with 50 c.c. of sterile saline and shaken with a few sterile beads for five minutes. This suspension is filtered through four layers of sterile gauze into a sterile centrifuge tube. If the patient has agglutinins for Brucella in his blood, 0.5 c.c. of his inactivated serum

is added to the filtrate. Diagnostic serum without preservative is used if the patient does not have agglutinins. This mixture is placed in the icebox overnight, and the next morning it is centrifuged at second speed for two minutes. The supernatant fluid is discarded, the sediment is resuspended in 10 c.c. of sterile saline and recentrifuged. This procedure is repeated three times. After the last centrifuging, the sediment is resuspended in 5 c.c. of beef infusion broth, pH 7.4. A loopful of this mixture is streaked over the surface of four eosin methylene blue and two liver infusion blood agar plates. One cubic centimeter of the suspension is put into each of two flasks containing 100 c.c. of beef infusion broth, pH 7.4. Half of the cultures are incubated in the presence of 10 per cent carbon dioxide.

The cultures are examined daily, after the first forty-eight hours' incubation. The colonies of *Brucella* resemble those of *S. dysenteriae* on the eosin methylene blue agar plates. These colonies are transferred to liver infusion blood agar slants. When there is sufficient growth, the organism is identified in the usual manner.

After the first forty-eight hours' incubation, the broth cultures are centrifuged, and the sediment is streaked over the surface of two liver infusion blood agar plates. Each of two flasks are inoculated with 1 c.c. of this sediment, and incubated for forty-eight hours, at which time the procedure is repeated. The transplants are incubated under the same condition as the original cultures. *Cultures of feces for Brucella should be kept for one week before they are reported as negative.*

Urine.—A fresh specimen of urine is centrifugalized at high speed for fifteen minutes. The supernatant fluid is discarded, and the sediment is used for culture. One-tenth cubic centimeter of the sediment is streaked over the surface of each of two liver infusion blood agar plates. One plate is incubated in the presence of 10 per cent carbon dioxide. When present, the organisms grow readily, producing typical colonies containing characteristic coccoid or bacillary forms. After forty-eight hours' incubation, if typical colonies are not present, transplants are made to blood agar plates at two- to three-day intervals. *The cultures are kept for ten days before they are reported as negative.*

Necropsy.—Cultures should be made as soon as possible after death. The surface of the liver, spleen, and kidneys is seared, and a small piece of tissue is removed with sterile scissors (a separate pair of scissors for each organ). If possible, axillary and mesentery lymph nodes are obtained. The small particles of tissue are washed through a series of three Petri dishes containing sterile saline, and then passed through the flame to remove any surface contaminants. The material is ground in a sterile mortar with sterile sand, to which are added 2 c.c. beef infusion broth. One-tenth cubic centimeter of this suspension is streaked over the surface of each of two liver infusion blood agar plates, and incubated in the usual manner. The lymph nodes are cultured as described previously.

The cultures are examined daily and transplants are made at two- to three-day intervals after the first forty-eight hours' incubation. *These cultures are kept for two weeks before they are reported as negative for Brucella.* Guinea pigs are inoculated and followed in the usual manner.

DISCUSSION

The cultures should be examined daily, and transplants of broth cultures should be made to fresh broth at two- to three-day intervals. A Gram's stain of the broth may not be very conclusive, unless the examiner is accustomed to looking at stained smears of the organism. When colonies on solid media first become visible to the naked eye, they appear as minute, transparent, colorless drops on the surface. *Transplants should be made to fresh agar at two- to three-day intervals even though there is no visible growth.* Brucella will not grow on dried agar, so, unless transplants are made to fresh, moist media, the colonies will not increase in size and the organism will disappear. When trying to isolate this group of organisms, it is very important to recognize growth when it first appears and to be able to distinguish between organisms and artifacts in the stained smear.

When organisms are present in small numbers, as in the blood of patients with the chronic form of brucellosis, and in lymph nodes, Brucella may be recovered occasionally from guinea pigs when the cultures are negative.

CONCLUSIONS

The cultural methods for the isolation of Brucella from various parts of the body are described in detail. Especial emphasis is laid upon the necessity of recognizing microscopically the very young forms of Brucella, and upon the study of the cultures for several weeks, if necessary. Disregard of these two factors may result in failure to demonstrate existing organisms.

REFERENCES

1. Huddleson, I. F.: Brucellosis in Man and Animals, The Commonwealth Fund, New York, 1939.
2. Poston, M. A.: Studies on Chronic Brucellosis. III. Methods Used in Obtaining Cultures, Pub. Health Rep. 53: 1, 1938.
3. Amoss, H. L., and Poston, M. A.: Cultivation of Brucella From the Stools and Bile—Further Observations, J. A. M. A. 95: 482, 1930.
4. Poston, M. A., and Parsons, P. B.: Isolation of Brucella From Lymph Nodes, J. Infect. Dis. 66: 86, 1940.
5. Poston, M. A., and Smith, D. T.: Successful Treatment of Brucella Meningitis With Immune Human Serum: Isolation of the Organism by a Modified Cultural Method, New England J. Med. 215: 369, 1936.
6. Amoss, H. L., and Poston, M. A.: Undulant (Malta) Fever—Isolation of the Brucella Organism From the Stools, J. A. M. A. 93: 170, 1929.

A NEW OBJECTIVE CIRCULATION TIME TEST (FLUORESC EIN METHOD)*

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A NEW method for estimating the speed of the circulating blood has been done on rabbits and on people. To date, the only reliable simple methods for determining complete circulation time are subjective in nature. A substance is injected into the antecubital vein of the arm, and the time is noted when the patient states that he tastes something bitter, burning, hot, etc., depending upon the substance used. Various substances have been suggested for determining the arm-to-tongue time. Decholin,¹ saccharin,² calcium gluconate,^{3,4} and magnesium sulfate⁵ have been used. One must take into account inaccuracies in such a method. It cannot be used in small children, comatose people, or anesthetized patients. It would, therefore, be more accurate to have an objective method whereby a dye could be injected into the arm and the time noted when the dye appeared in the tongue, eye, etc.

I decided to use a very fluorescent substance, because even in high dilutions the color could be noted by the use of an ultraviolet machine with a filter (Woods filter). The ultraviolet machine was a water-cooled mercury quartz lamp (Kromayer lamp). Young male white rabbits, weighing 6 to 10 pounds, were used. With the rabbit's head projecting through an opening in a box, one assistant injected the fluorescent substance intravenously into the marginal vein of the ear, while another started the stop watch. Then the light switch was turned off, making the room completely dark. The light from the ultraviolet machine was directed toward one of the rabbit's eyes, and the conjunctiva and iris were noted to have a purplish hue. Suddenly a yellow color appeared on the inner margins of the eyelids (conjunctiva), and bright yellow vertical lines spreading into tiny branches were noted in the iris. This occurred simultaneously with a yellow color on the mucous membranes of the lips. There would thus be a sudden sharp end point to stop the stop watch. The time that elapsed between the rapid intravenous injection into the marginal vein of the ear, the passage of the substance through the right side of the heart, to the lungs, back to the left side of the heart, out through the aorta and to the periphery where it was best noted in the eye, was observed.

In rabbits I used the following fluorescent substances: 1 per cent acridine orange, 1 per cent flavophosphine, 1 per cent phosphine, 0.5 per cent acriflavine, 0.4 per cent indigo carmine, riboflavin, and 1, 5, and 10 per cent fluorescein.

RESULTS

2 c.c. 1 per cent acridine orange—Usually faint yellow reaction, but occasionally good yellow reaction.

1 c.c. 1 per cent flavophosphine—Poor yellow reaction.

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- 2 c.c. 1 per cent flavophosphine—Good yellow reaction.
- 2 c.c. 1 per cent phosphine—Fair yellow reaction.
- 2 c.c. 0.5 per cent acriflavine—Good yellow reaction.
- 2 c.c. 0.4 per cent indigo carmine—No color reaction.
- 2 c.c. riboflavin—Very faint yellow reaction.
- 2 c.c. 1 per cent fluorescein—Very good yellow reaction.
- 1 c.c. 5 per cent fluorescein—Very good yellow reaction.
- 1 c.c. 10 per cent fluorescein—Brilliant yellow reaction (best).

Therefore, fluorescein gave the best color reaction, being the most intense yellow when noted in the eye. Since sodium fluorescein is more soluble than fluorescein, the former was used to make up a 10 per cent solution. Acriflavine appeared to be the next best.

The circulation time test was done in over 70 instances, although the same rabbits were used on successive days or weeks. The same rabbit could even be used about one hour after the test, since much of the dye would disappear from the eye in that time. The circulation time in rabbits averaged between five and six seconds. Occasionally, it would be as low as $4\frac{2}{5}$ seconds or as high as seven seconds.

In rabbits, 2 c.c. of 5 per cent sodium fluorescein injected intravenously gave a slight yellow reaction in the inner margins of the eyelids at the end of eight seconds, using ordinary daylight and no ultraviolet machine. However, the end point was not sharp. Therefore, for this experiment it is advisable to have an ultraviolet machine, a filter suited for fluorescence, and a room that can be made dark by turning off the light switch.

A 1 c.c. tuberculin syringe and a No. 24 gauge needle were used in the rabbits, since this was found to consume less than one-half second for intravenous injection.

The 10 per cent sodium fluorescein solution in distilled water was autoclaved for fifteen minutes, a sample was checked for sterility and toxicity by inoculation into a rabbit, and then used for intravenous injection in human beings. The circulation time was then noted to be $11\frac{1}{2}$ seconds, 12 seconds, etc., in normal people. This was done in seven cases. There were no toxic reactions noted and no change in temperature, pulse rate, and respiration. A 5 c.c. syringe and a No. 21 or 18 gauge needle were used in man, with 4 c.c. of 10 per cent sodium fluorescein injected intravenously. In several additional cases, 3 c.c. of 20 per cent sodium fluorescein was used intravenously and gave even a better color reaction. There were no untoward reactions. By pulling the lower eyelid down and directing the ultraviolet light toward the conjunctiva after the substance was injected intravenously, a sharp yellow end point could be noted.

SUMMARY

A new objective circulation time test is presented, and after using various fluorescent substances, it was found that in rabbits the following technique was best. Using a 1 c.c. tuberculin syringe and a No. 24 gauge needle, 1 c.c. of 10 per cent sodium fluorescein was injected into the marginal vein of the ear. The

stop watch was started, the light was switched off in the room, the ultraviolet light plus filter was directed toward the rabbit's eye, and the stop watch was stopped immediately upon noting a brilliant yellow color in the iris of the eye. Simultaneously, the conjunctiva along the inner margin of the eyelids would become a brilliant yellow.

The average circulation time in rabbits was five to six seconds.

A similar test is being done in human beings, using 4 c.c. of 10 per cent sodium fluorescein or 3 c.c. of 20 per cent sodium fluorescein.

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REFERENCES

1. Tarr, L., Oppenheimer, B. S., and Sager, R. V.: Circulation Time Determined by Use of Sodium Dehydrocholate, *Am. Heart J.* 8: 766, 1933.
2. Fishberg, A. M., Hitzig, W., and King, F. H.: Measurement of Circulation Time With Saccharin, *Proc. Soc. Exper. Biol. & Med.* 30: 651, 1933.
3. Goldberg, S. J.: Use of Calcium Gluconate as a Circulation Time Test, *Am. J. M. Sc.* 192: 36, 1936.
4. Baer, S., and Slipakoff, B. G.: Measurement of Circulation Times and Agents Used in Their Determination, *Am. Heart J.* 16: 29, 1938.
5. Bernstein, M., and Simpkins, S.: Use of Magnesium Sulphate in Measurement of Circulation Time, *Am. Heart J.* 17: 218, 1939.

2806 FRANKFORD AVENUE

CHEMICAL

THE PHOTOMETRIC DETERMINATION OF SUGAR IN BIOLOGICAL FLUIDS BY FERRICYANIDE REDUCTION*

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WITH the advent of photometry or "photoelectric colorimetry" into biochemical analysis, numerous papers have been published on the photometric determination of sugar. Yet none of these methods has been so successful from the point of speed, precision, and reproducibility as to aid in supplanting the visual methods used at present. We feel that this paper meets such a need for the routine clinical analysis of sugar in biological fluids.

Photometric methods for sugar are in the main adaptations of existing copper or ferrieyanide methods. Of the copper methods, modifications of the original Folin-Wu¹ have been the most popular. This method has been investigated by Sanford, Sheard, and Osterberg,² and by Fiorentino and Giannettasio.^{3, 4} We have carefully investigated both the Folin-Wu and the Benedict⁵ methods with a view to their possible adaptation to the photoelectric colorimeter. It was found impossible to obtain checks on runs performed from day to day, using the same set of reagents. Accurate results could be obtained only if a sufficient number of standards were run with each unknown, or set of unknowns, to allow for the plotting of a new calibration curve. An inherent advantage of ferrieyanide methods is the greater stability of the reduction product, ferroeyanide, over the reduction products of the copper reagents. Therefore, photometric ferrieyanide methods were then investigated. Hoffman⁶ has described an adaptation of the ferrieyanide fading method of Hawkins and Van Slyke.⁷ The method has been found to give good results which are reproducible from day to day. The disadvantages of the method are: (1) an additional quantitative measurement is required, i.e., the ferrieyanide in addition to the filtrate; (2) the spread is small, being only 30 divisions for a difference of 200 mg. of glucose per 100 c.c.; (3) the method is least sensitive at low sugar values and the most sensitive at high values.

The Folin-Malmros⁸ ferrieyanide colorimetric method has been found to be particularly suited for microsugar methods because of the intense blue colors obtained for small amounts of biological fluids. This method has been adapted for the photoelectric colorimeter by Jourdonais,⁹ using the original procedure

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except for a longer heating time. This author finds that the colors produced do *not follow Beer's law* and give incomplete recoveries for added amounts of pure glucose. A special correction curve is required to obtain correct results. Marenzi and Villalonga¹⁰ also have applied the Folin method to the photoelectric colorimeter. An advantage of their method is the substitution of lithium and ammonium tartrate in place of gum ghatti in the ferric iron reagent. The method, however, uses sodium cyanide in the alkaline reagent. (The undesirable effects of sodium cyanide will be noted later.) The method has also been investigated by Evelyn,¹¹ who increases the range of readings by leaving out the sodium cyanide from the carbonate reagent and runs his calibration curve in the presence of the tungstic acid solution used for the precipitation. The method still makes use of the ferric iron-gum ghatti reagent which gives variable blanks.

A visual method recently described by Klendshoj and Hubbard¹³ does not use sodium cyanide, and substitutes for the gum ghatti an emulsifying agent (Duponol) which gives negligible blanks, yet stabilizes the Prussian blue solutions over long periods of time.

The last method, with several important modifications, has been adapted by us for purposes of photoelectric colorimetry. For routine sugar analyses the method has been found to give values that compare favorably in precision with the Hagedorn-Jensen¹⁴ procedure and in speed of determination with the original Folin-Wu procedure.

EXPERIMENTAL

A. Reagents.—(1) Ferrieyanide reagent: 0.1 per cent of pure, crystalline, potassium ferrieyanide (prepared free of ferrocyanide according to Folin as given by Peters and Van Slyke¹⁵) was dissolved in a 3 per cent solution of sodium carbonate. The appropriate amount of ferrieyanide was weighed on an analytical balance, made up to volume in a volumetric flask with 3 per cent sodium carbonate solution, and allowed to stand in a dark closet for several days. Any precipitate formed was removed by filtering the solution into a glass-stoppered, amber reagent bottle before using.

(2) Ferric iron reagent: Into a liter beaker weigh 5 Gm. of ferric sulfate (Mallinckrodt—analytical grade) and dissolve with stirring in about 500 ml. of distilled water. (Some difficulty may be encountered in bringing the ferric salt into solution, but HEAT MUST NOT BE USED if reproducible results are desired with each fresh batch of the reagent prepared.) Add slowly 75 ml. of 85 per cent phosphoric acid (reagent grade), stirring constantly. A precipitate or cloud forms, which quickly dissolves with the further addition of the phosphoric acid. An absolutely water-clear solution should now be obtained which is diluted to 1 liter with distilled water. Now add 16 Gm. of "Duponol" P.C. (an emulsifying agent sold by E. I. Du Pont De Nemours and Co., Fine Chemicals Division, New York, N. Y.) in small amounts, stirring constantly until complete solution takes place. Remove all traces of turbidity by allowing the solution to stand in an incubator at 37° C. overnight. Add 5 ml. of concentrated sulfuric acid (reagent grade, sp. gr. 1.84) to repress the hydrolysis of the ferric salt. The solution is now ready for use and will remain clear almost indefinitely if kept at room temperature when not in use.

(3) Tungstic acid* solution for the preparation of the Folin-Wu filtrate: Solution A: 6 ml. of concentrated sulfuric acid (reagent grade, sp. gr. 1.84)—diluted to 2 liters with distilled water in a volumetric flask. Solution B: 111 Gm. of sodium tungstate ($\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$) are dissolved in a liter of solution.

For use, 8 parts of solution A are mixed with 1 part of solution B. This should be prepared fresh at least once weekly.

For whole blood, serum, or plasma: one part of whole blood, etc., is mixed with 9 parts of the tungstic acid mixture. Shake. Allow to stand until the mixture turns chocolate brown, and filter through a Whatman No. 1 or No. 2 filter paper.

(4) Glucose standard solutions: (a) A solution of 100 mg. per 100 c.c. of pure, dry, anhydrous Bureau of Standards glucose was prepared in 0.25 per cent benzoic acid solution. (b) A solution of glucose containing 2 mg. per 100 c.c. was prepared by dilution of standard (a) with 0.25 per cent benzoic acid. 1 ml. is equivalent to 100 mg. of glucose per 100 c.c. (c) A solution of glucose containing 5 mg. per 100 c.c. was prepared as in solution (b). 1 ml. is equivalent to 250 mg. of glucose per 100 c.c. (d) A solution containing 10 mg. per 100 c.c. was prepared as in (b). 1 ml. is equivalent to 500 mg. of glucose per 100 c.c.

B. *Selection of Color Filter.*—The proper color filter was selected from spectrophotometric data, obtained over a range of glucose concentrations varying from 0 mg. to 400 mg. per 100 c.c., by means of a Coleman double monochromator spectrophotometer. Careful study of these data showed that Beer's law does *not* apply for any of the wave lengths studied. The most satisfactory transmission band was found to be in the neighborhood of 520 $m\mu$. Six millimeters of a Sextant Green filter—Corning No. 401 (470 to 580 $m\mu$ transmission limits) were, therefore, used for all photometric determinations in this paper.

C. *Description of Instrument and Accessories.*—The instrument† used was essentially the same as that described by Evelyn¹⁰ with several minor modifications so as to make it more suitable and convenient for the routine clinical analysis.

The diameter of the tube holder was made smaller so that it would accommodate ordinary pyrex test tubes (16 mm. by 150 mm.) instead of the larger soft glass tubes described by Evelyn. The tubes used in these determinations were carefully selected from large batches of pyrex tubes in the manner described by Evelyn¹² and were accurately calibrated for 5 ml. and 10 ml. volumes of solution. The determinations were performed directly in the tubes, and the colors were developed and read in the instrument *without* transfers.

The methods as described can be used without change with any standard photoelectric colorimeter now on the market that will accommodate a 10 ml. volume (or less) of solution. With minor adjustments in the volumes of the sample and the reagents used, the method is readily adaptable to any type of photoelectric instrument. For example, by using two and one-half times the

*Routine determinations for urea nitrogen are performed in this laboratory by adding a concentrated urease solution to the whole blood previous to the protein precipitation. A tungstic acid solution stronger than usual is required to precipitate both the excess urease and the blood proteins in order to obtain water-clear filtrates.

†The instrument was designed and built by Mr. Sidney X. Shore, Radio Engineer of Station WNYF, Fire Department of the City of New York.

quantities of sample and reagents given below, the procedure may be performed in regular Folin-Wu sugar tubes, with a final dilution to the 25 ml. mark. The color may then be read by means of transfer with photoelectric instruments which employ optical cells or ungraduated tubes.

Procedure.—(1) Preparation of the calibration curve: Two-tenths milliliter of a 1:4 dilution* of the tungstic acid mixture described above was pipetted into each of the pyrex photoelectric colorimeter tubes. Known amounts of the glucose standard solutions were then pipetted into the appropriate tubes using calibrated volumetric pipettes. One milliliter of the alkaline ferrieyanide reagent was added with a 5 ml. serologic pipette to each tube. Distilled water was added to bring the total volume to about 4 ml. The tubes were heated in a boiling water bath for eight minutes, and then cooled in running water. Five milliliters of the ferrie iron-Duponol reagent were then added and the contents were mixed. Distilled water was then added up to the 10 ml. calibration mark,†

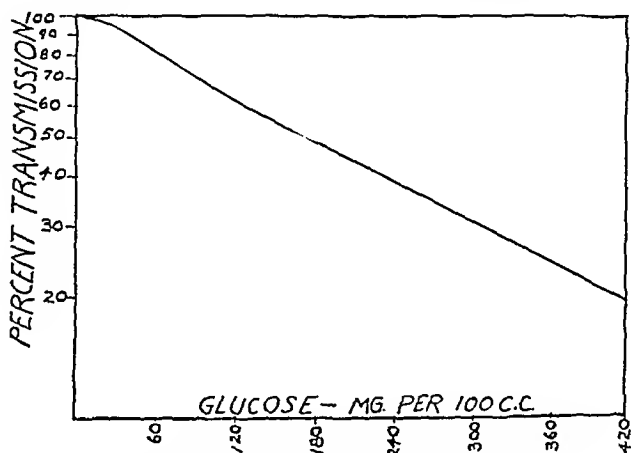


Fig. 1.—Calibration curve for standard glucose solutions for the colorimetric ferrieyanide method. Corning filter No. 401-6 mm.

and the contents were mixed by inversion. The outside surface of the tubes was carefully washed and wiped dry with gauze or lens paper. The tubes were read in the instrument at various time intervals up to sixty minutes against a reagent blank set at 100 per cent transmission. The data for the thirty-minute interval were plotted on semi-logarithmic graph paper and are shown in Fig. 1. The reagent blank was constant and has a transmission of about 98 per cent against distilled water.

A summation of the data obtained indicates: (a) The curve follows Beer's law only between 100 mg. and 400 mg. per 100 c.c. of glucose with any degree of precision. Therefore, a calibration curve, or a table compiled from such a curve, is a necessity if accurate results are to be obtained. (b) Fluctuations obtained in the galvanometer readings for standard glucose solutions are greatest in values below 100 mg. per 100 c.c., and least in values of more than 100 mg. per

*The reason for the 1:4 dilution of the tungstic acid mixture will be discussed later in the paper.

†If foaming occurs, a wire dipped into caprylic alcohol may be touched to the surface of the solution in order to facilitate dilution to the mark.

100 c.c. (c) Good results can be obtained even for low sugar values if the readings are taken between thirty and forty minutes after the addition of the ferric iron reagent. (d) The checks obtained in the triplicate samples with the same glucose concentrations are excellent.

Readings on all further determinations in this paper were, therefore, made between thirty and forty minutes, and the calibration curve (or a table compiled from such a curve) was used to obtain the correct glucose values.

Analytical Determinations.—Hiller, Linder, and Van Slyke¹⁷ have pointed out that the method of Hagedorn-Jensen can be applied to the determination of sugar in Folin-Wu tungstic acid blood filtrates with good results. If tungstic acid filtrate is used in the Hagedorn-Jensen method (in place of the zinc filtrate) from a theoretical point of view, the method should give results that are in perfect agreement with the colorimetric ferrieyanide method we used. Both methods use a similar reducing reagent, i.e., ferrieyanide in sodium carbonate solution, so that the reaction products formed in each of the two methods is the same. The only REAL difference between the two methods is that the Hagedorn-Jensen method determines the excess ferrieyanide (which requires an additional quantitative measurement) by means of an iodometric titration using starch as the indicator, while our method determines the ferrieyanide formed directly by means of the Prussian blue color using a photoelectric colorimeter. Fujita and Iwatake¹⁸ have found that the Hagedorn-Jensen method does not give strictly proportional reduction values over a wide range of glucose values, and for this reason they advocate the use of their own phosphate buffer. To offset this difficulty, since the original Hagedorn-Jensen was used in these determinations, the colorimetric determinations were always run first. Aliquots of filtrate were then selected so that the Hagedorn-Jensen values would all fall within a very limited range. As an added precaution, a set of blanks and a set of standards were run with each series of Hagedorn-Jensen titrations. With these added precautions, the two methods were found to give strictly comparable results.

(1) *Whole blood determinations:* Tungstic acid filtrate (1:10) of whole blood was prepared in the manner described under the preparation of the reagents. Two-tenths milliliter samples of the 1:10 filtrate or 2 ml. samples of 1:100 filtrate (where more accurate results are desired) were pipetted into the appropriate colorimeter tubes, using calibrated volumetric pipettes. One milliliter of the alkaline ferrieyanide solution was then added, and the rest of the determination was carried out as given in the foregoing procedure. The tubes were read after standing for a thirty-minute interval. These determinations were run in triplicate for the purpose of comparison with the Hagedorn-Jensen procedure. A set of glucose standards was run with each set of unknowns. Recoveries were run on the whole blood samples by pooling many bloods together, so that a large quantity of blood was available. A sample of this pooled blood was taken off for analysis in the manner already described. An accurately weighed quantity of Bureau of Standards glucose was placed in a volumetric flask (100 ml.), the glucose was dissolved in the blood sample, and the solution was diluted to mark with the whole blood. Tungstic acid filtrates of this blood

were then prepared, and the sugar then present was determined. The difference between the two values obtained gave the recovery of pure glucose by this method.

The values obtained by this method for whole blood, their comparison with the Hagedorn-Jensen (also run in triplicate), and the recoveries obtained by this method are given in Table I.

TABLE I
DETERMINATION OF SUGAR IN BIOLOGICAL FLUIDS
(Every figure in table represents the average of three or more determinations.)

SAMPLE NO.	PHOTOMETRIC METHOD	HAGEDORN-JENSEN METHOD	GLUCOSE ADDED	GLUCOSE PHOTOMETRIC	
				CALCULATED	FOUND
MILLIGRAMS PER 100 C.C.					
Whole blood					
1	221	214			
2	71	75			
3	100	102			
4	87	85	200	287	279
5	54	51	100	154	153
6	124	120	50	174	171
Plasma					
1	88	88			
2	48	49			
3	182	179	100	282	279
4	69	70	200	269	268
Serum					
1	54	56			
2	47	50			
3	49	50	100	149	148
4	62	60	200	262	265
Cerebrospinal fluid					
1	54	50			
2	128	133			
3	87	85			
4	64	63			
Urine					
1 (Normal)	0				
2 (Normal)	0		200	200	200
3 (Normal)	0		500	500	496
4	560	547			
5	1,250	1,220			

(2) *Plasma determinations:* Run as for whole blood. See Table I for the results obtained.

(3) *Serum determinations:* Run as for whole blood. See Table I for results obtained.

(4) *Spinal fluid determinations:* Determinations were performed directly on the spinal fluid samples without protein precipitation. The samples were diluted (1:6) with 0.85 per cent sodium chloride for the Hagedorn-Jensen determinations, and then these samples were diluted (1:10) again (or a total dilution of 1:60) for the colorimetric determinations. Because of the small amounts of material available, no recoveries were run. For routine analysis, 0.5 ml. of a 1:10 dilution of the original fluid may be used. The result obtained from the curve is then divided by 2.5 to obtain the correct glucose value.

The results obtained in these runs and their comparison with the Hagedorn-Jensen method are given in Table I.

(5) *Urine determinations*: The samples were prepared in the manner described in Peters and Van Slyke,¹⁹ using Lloyd's reagent. A rough quantitative test was performed on each urine, and the sample was diluted in such a manner as to give values between 0.1 mg. and 0.2 mg. of sugar for the Hagedorn-Jensen runs, and 0.01 mg. and 0.02 mg. of sugar for the colorimetric determinations. Recoveries were run in the same manner as described under whole blood, using normal (or sugar-free) urine. The blank consisted of 2.0 ml. of a 1:1,000 dilution of this urine.

The results obtained in these runs and their comparison with the Hagedorn-Jensen method are given in Table I.

DISCUSSION

A careful physicochemical study of the reducing action of glucose on ferri-cyanide has been carried out by Wood,²⁰ using potentiometric measurements. We have investigated, by means of the photoelectric photometer, the effect of variations of concentrations of the reagents used in the above procedure (following the criteria laid down by Wood). In general, with one important exception (i.e., the use of sodium cyanide), our findings confirm those of Wood.

The concentrations of glucose used in this method (0 mg. to 0.08 mg. glucose or 0 mg. to 400 mg. per 100 c.c.) were chosen for the following reasons:

(a) A 0.2 ml. aliquot of a 1:10 Folin-Wu filtrate could be easily and accurately measured by means of calibrated mark to mark pipettes.

(b) It is *not* desirable to take readings below 20 per cent transmission, since the instrument is relatively insensitive in that region; i.e., large changes of glucose concentration produce relatively small changes of the transmission readings.

(c) The emulsifying agent (Duponol) can hold only a limited amount of Prussian blue in solution over long periods of time (minimum forty-five minutes). Concentrations of glucose greater than 400 mg. per 100 c.c. cause "cracking" or flocculation of the Prussian blue precipitate in relatively short periods of time.

The amount of potassium ferri-cyanide used in this method has been found to be sufficient to take care of the reducing action between 0.10 mg. and 0.12 mg. of glucose, i.e., 500 mg. to 600 mg. per 100 c.c. However, determinations of fluids with sugar concentrations of more than 400 mg. per 100 c.c. should be repeated, using smaller aliquots of the sample. The effect of the benzoic acid used to preserve the sugar standards caused no noticeable reduction of the ferri-cyanide. In order to duplicate as closely as possible the conditions under which glucose is reduced in a blood filtrate, the average concentration of tungstic acid remaining in blood filtrate after precipitation of the proteins was determined gravimetrically.²¹ This quantity of tungstic acid was then added to the glucose standards. It was noted that the alkalinity of the ferri-cyanide affects the rate and amount of reduction considerably. Experimental results obtained showed that a ferri-cyanide reagent containing 3 Gm. of sodium carbonate per 100 ml. of ferri-cyanide solution (0.1 per cent) gave optimum results. Experimental runs made to find the minimum time of heating showed that at least five minutes was necessary for concentrations of sugar below 400 mg. per 100 c.c. Therefore, a

heating time of eight minutes was selected to give a safe margin of time for the complete reduction of the ferrieyanide by glucose. The use of sodium cyanide, as used in the original Folin reagent, was found to be undesirable, because it reduces the range of the method by one-half, and it also seriously affects the reproducibility of results for the reason that the amount of ferrieyanide produced increases with the time of heating up to about forty-five minutes when it begins to level off.

That precise and reliable results can be obtained by this method is shown by the analytical data given in this paper for the various biological fluids. Results obtained by the photometric method are in close agreement with those of the Hagedorn-Jensen method, and the recoveries obtained for added glucose are excellent. The method, as given here, has been used in this laboratory for routine clinical analysis for more than one year with uniformly excellent results. Blanks, and 100 mg. and 200 mg. per 100 c.c. standard glucose solutions have been run daily for that period, with an average error of about ± 2 per cent. In the course of performing these routine determinations and doing the analytical work of this paper, new batches of each reagent were made up at relatively frequent intervals. We were able to check constantly the points of our original calibration curve.

The method, in addition to being more precise, reliable, and economical of material than the old Folin-Wu procedure proved to be much faster for a large number of sugar determinations. The only quantitative measurement required is that of the 0.2 ml. of the 1:10 Folin-Wu filtrate. The sodium carbonate-ferrieyanide solution is pipetted as one reagent, and all the succeeding operations are performed in the individual pyrex tubes without any transfers. The colors developed are read in these same tubes at the rate of about five a minute.

Some objections to the method may arise from the fact that a long waiting time (thirty minutes) is required to allow for full color development before the colorimeter readings are taken. This would be of particular importance in those laboratories where only a few determinations are performed at one time, or in the case of an emergency determination. For emergency determinations, results of sufficient accuracy may be obtained by taking the readings at a ten or twenty minute-time interval, or if more accurate results are desired, additional calibration curves may be drawn for these time intervals.

In agreement with Somogyi²² we have found that ferrieyanide methods give sugar values that average about 20 mg. per 100 c.c. higher for tungstic acid filtrate than those obtained by the copper methods of Shaffer-Hartmann²³ or Benedict. These copper methods give "true sugar" values in that they are not affected by the nonglucose reducing substances present in the tungstic acid filtrate.

We have confirmed the observations of Hoffman, and of Klendshoj and Hubbard, that the colorimetric ferrieyanide method gives results which are in agreement with those obtained by the old Folin-Wu procedure. The normal values for sugar obtained by this method are practically the same as those given by the old Folin-Wu procedure, i.e., from 85 mg. to 115 mg. per 100 c.c. of blood sugar.

SUMMARY

A photometric ferrieyanide method is described for the rapid, precise determination of sugar in small amounts of biological fluids, e.g., 0.2 ml. of a (1:10) blood filtrate. Careful studies of the factors influencing the reduction of ferrieyanide by glucose were made in order to determine optimum conditions and concentrations of reagents. Negligible and constant blanks were obtained by the substitution of "Duponol" for gum ghatti. Reproducible values for standard solutions were obtained for day to day runs even when new reagents were used. The methods gave results for biological fluids that were in agreement with those obtained by the Hagedorn-Jensen procedure and gave excellent recoveries of added glucose.

REFERENCES

1. Folin, O., and Wu, H.: A Simplified and Improved Method for the Determination of Sugar, *J. Biol. Chem.* 41: 367, 1920.
2. Sanford, A. H., Sheard, C., and Osterberg, A. E.: The Photometer and Its Use in a Clinical Laboratory, *Ann. J. Clin. Path.* 3: 403, 1933.
3. Fiorentino, M., and Giannettasio, G.: The Photometric Determination of Blood Sugar by the Method of Folin and Wu, *Diag. e tec. di lab.* 10: 401, 1939.
4. Fiorentino, M., and Giannettasio, G.: Photometric Blood Sugar Determinations by the Folin-Wu Method. A New Source of Error, *J. LAB. & CLIN. MED.* 25: 866, 1940.
5. Benedict, S. R.: The Analysis of Whole Blood. II. The Determination of Sugar and of Saccharoids, *J. Biol. Chem.* 92: 141, 1931.
6. Hoffman, W. S.: A Rapid Photoelectric Method for the Determination of Glucose in Blood and Urine, *J. Biol. Chem.* 120: 51, 1937.
7. Hawkins, J. A., and Van Slyke, D. D.: A Time Method for the Determination of Reducing Sugars and Its Application to Analysis of Blood and Urine, *J. Biol. Chem.* 81: 459, 1929.
8. Folin, O., and Malmros, H.: An Improved Form of Folin's Micro Method for Blood Sugar Determinations, *J. Biol. Chem.* 83: 115, 1929.
9. Jourdonais, L. F.: Standardization of the Folin-Malmros Micro Blood Sugar Method for Capillary and Venous Blood and Its Adaption to the Photoelectric Colorimeter, *J. LAB. & CLIN. MED.* 23: 847, 1938.
10. Marcelli, A. D., and Villalunga, F.: A Folin-Malmros Method of Blood Sugar Determination-Photometric Application, *Rev. Soc. argent. de biol.* 15: 246, 1939.
11. Evelyn, K. A.: Evelyn Photoelectric Colorimeter. Notes on Operation, Philadelphia, 1939, Rubicon Co., page 21.
12. Evelyn, K. A.: *Ibid.*, Philadelphia, 1939, Rubicon Co., pp. 6, 7.
13. Klendshoj, N. C., and Hulbard, R. S.: A Method for the Determination of Sugar in Small Amounts (0.02 c.c.) of Blood, *J. LAB. & CLIN. MED.* 25: 1102, 1940.
14. Hagedorn, H. C., and Jensen, B. N.: Zur Mikrobestimmung des Blutzuckers mittels ferrieyanide, *Biochem. Ztschr.* 135: 46, 1923.
15. Peters, J. P., and Van Slyke, D. D.: Quantitative Clinical Chemistry, Baltimore, 1932, Williams & Wilkins Co., Vol. 2, Methods, page 462.
16. Evelyn, K. A.: A Stabilized Photoelectric Colorimeter With Light Filters, *J. Biol. Chem.* 115: 63, 1936.
17. Hiller, A., Linder, G. C., and Van Slyke, D. D.: The Reducing Substances of the Blood, *J. Biol. Chem.* 64: 625, 1925.
18. Fujita, A., and Iwatake, D.: Bestimmung des echten Blutzuckers ohne Hefe, *Biochem. Ztschr.* 242: 43, 1931.
19. Peters, J. P., and Van Slyke, D. D.: *Ibid.*, Baltimore, 1932, Williams & Wilkins Co., page 452.
20. Wood, W. B., Jr.: A Preliminary Physico-Chemical Study of the Reducing Action of Glucose, *J. Biol. Chem.* 110: 219, 1935.
21. Knorre, G. V.: Ueber ein neues Verfahren Zur Bestimmung von Wolfram, *Ber. chem. Ges.* 38: 783, 1905.
22. Somogyi, M.: A Method for the Preparation of Blood Filtrates in the Determination of Sugar, *J. Biol. Chem.* 86: 655, 1930.
23. Shaffer, P. A., and Hartmann, A. F.: II. Methods of the Determination of Reducing Sugars in Blood, Urine, Milk, and Other Solutions, *J. Biol. Chem.* 45: 365, 1921.

THE DETERMINATION OF BLOOD LIPIDS IN BLOOD DYSCRASIAS*

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THE objects of this paper are to compare the values for total blood plasma lipids in blood dyscrasias obtained from the usual alcohol-ether extracts and from petroleum-ether extracts, and to study the nature of the nonlipid contaminants in these extracts.

Pernokis and Freeland^{1, 2} reported elevated total lipid values obtained gravimetrically from alcohol-ether extracts in cases of blood dyscrasias. Their work was well in progress when Christensen³ and almost simultaneously, Van Slyke and Foleh,⁴ reported that nonlipid contaminants were present in the alcohol-ether extracts and also, to a lesser degree, in the petroleum-ether extracts. They also clearly demonstrated that the bulk of these contaminants were urea and chlorides.

We approached the problem in two ways which will be described in turn. The first method was to compare the total lipid values obtained from alcohol-ether extracts with those obtained from petroleum-ether extracts.

Blood plasma was extracted with the usual alcohol-ether mixture. Aliquots of this extract were carefully evaporated to dryness and weighed. This value appears in Table I as "Total Lipids" under the heading "Alcohol-Ether Extract." This alcohol-ether soluble residue was then extracted five times with 5 c.c. portions of petroleum-ether extract. The combined petroleum-ether extract was then carefully evaporated to dryness and weighed. This value appears in Table I as "Total Lipids" under the heading "Petroleum-Ether Extract."

To obtain the values for "Total N as Urea" in Table I, aliquots of both the alcohol-ether and petroleum-ether extracts were subjected to micro-Kjeldahl analysis. The values obtained for total nitrogen were converted to their equivalents in terms of urea because urea is the form in which practically all of this nitrogen is present. The values for "Corrected Total Lipids" in Table I are the values obtained by subtracting the "urea" values from the "Total Lipids" values.

A comparison of the values for "Corrected Total Lipids," as shown in Table I, reveals that only from 46 to 72 per cent of the materials soluble in the alcohol-ether mixture from cases of myelogenous leucemia are soluble in petroleum-ether.

The second method of approach to the problem was an attempt to determine the composition of the material, which is soluble in the alcohol-ether mixture but insoluble in petroleum-ether extract. The procedure used was to use mixed

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alcohol-ether extracts from blood dyscrasias. The alcohol-ether mixture was carefully evaporated and the dry residue was weighed. The weight of this alcohol-ether soluble material was 0.1605 Gm. It was then extracted five times with 5 c.c. portions of petroleum ether. The petroleum ether was carefully evaporated. The dry residue weighed 0.0733 Gm. This is only 45.7 per cent of the weight of the alcohol-ether soluble substances. The petroleum-ether insoluble material (0.0872 Gm.) was dissolved in 100 c.c. of distilled water. This aqueous solution was used to determine chlorides as sodium chloride, urea, uric acid, creatinine, and total reducing sugars. The results are shown in Table II.

TABLE I

DIAGNOSIS	ALCOHOL-ETHER EXTRACT			PETROLEUM-ETHER EXTRACT			
	TOTAL LIPIOS (GM.)	TOTAL N AS UREA PER 100 C.C.	CORRECTED TOTAL LIPIOS PLASMA	TOTAL LIPIOS (GM.)	TOTAL N AS UREA PER 100 C.C.	CORRECTED TOTAL LIPIOS PLASMA	% PETROLEUM ETHER SOLUBLE
1. Pernicious anemia	1.270	0.053	1.217	0.856	0.021	0.835	68.6
2. Pernicious anemia	1.153	0.040	1.113	0.680	0.027	0.653	58.6
3. Acute myelogenous leucemia	1.293	0.058	1.235	0.933	0.044	0.889	71.9
4. Chronic myelogenous leucemia	1.400	0.048	1.352	0.666	0.043	0.623	46.0
5. Cholesterolemia	4.733	0.114	4.588	4.460	0.088	4.372	95.4
6. Leucemia cutis	1.400	0.072	1.327	0.800	0.034	0.766	57.7
7. Lymphatic leucemia	1.466	0.050	1.416	Accidentally lost			---
8. Uremia	2.133	0.167	1.966	1.130	0.157	0.973	40.4
9. Lymphatic leucemia	1.353	0.050	1.303	0.633	0.026	0.607	40.5
10. Chronic myelogenous leucemia	1.300	0.077	1.223	0.766	0.028	0.738	60.3

TABLE II

Sodium chloride	53.00 mg. or 60.8 per cent of residue
Uric acid	0.25 mg. or 0.3 per cent of residue
Creatinine	0.42 mg. or 0.5 per cent of residue
Urea	33.00 mg. or 37.8 per cent of residue
Reducing sugars	0.00 mg.
Total accounted for	86.67 mg. or 99.4 per cent of residue
Amount insoluble in petroleum ether	0.0872 Gm.
Amount recovered	0.0867 Gm.
Amount unaccounted for	0.0005 Gm.

The petroleum-ether insoluble contaminants consisted of 60.8 per cent of chlorides calculated as sodium chloride and 37.8 per cent of urea. These two contaminants accounted for 98.6 per cent of the total. Uric acid and creatinine accounted for 0.8 per cent, leaving 0.6 per cent unaccounted for. No reducing sugars were demonstrated to be present.

The petroleum-ether soluble material (0.0733 Gm.) was redissolved in petroleum ether and washed five times with 5 c.c. portions of water. The petroleum-ether was carefully evaporated, and the residue was reweighed. The loss of weight was 0.0087 Gm. of material dissolved in the water. This represents a loss of 11.8 per cent of the total petroleum-ether soluble material. The complete nature of this material has not yet been determined, but some of it is urea and chlorides. The problem is being further investigated.

DISCUSSION

The total corrected petroleum-ether soluble lipid values in two patients with pernicious anemia were 69 and 59 per cent of the values obtained for the alcohol-ether extract. In three patients with myelogenous leucemia, the total corrected petroleum-ether soluble lipid values ranged from 46 to 72 per cent of the values obtained for the alcohol-ether extract. The corrected petroleum-ether extraction values in three persons with lymphatic leucemia were 46 to 58 per cent of the alcohol-ether values. In one patient with uremia the petroleum-ether soluble material showed 49 per cent of the value obtained with the alcohol-ether extraction. The most interesting results were obtained in one person with hypercholesterolemia which showed a very marked increase in the total lipids of the blood with the alcohol-ether extraction method, and continued to show 95 per cent of that value in the petroleum-ether extract. This patient showed a tremendous increase in the fatty acids of the blood, values being as high as 2.758 Gm. Due to the unusual character of the lipids in this blood, the results were checked by three different chemists and found to agree.

CONCLUSION

Total lipid determinations done with the alcohol-ether extraction method show values which are much higher than the values obtained with the petroleum-ether extraction method. The values obtained with the petroleum-ether extraction varied from 46 to 95 per cent, with an average of 60.6 per cent of the total lipid values obtained with the alcohol-ether extraction.

The petroleum-ether insoluble material extracted with the lipids in the alcohol-ether extraction was analyzed and showed to contain 61 per cent of sodium chloride, and 38 per cent of urea. The other 1 per cent was present in the form of uric acid and creatinine. No reducing sugars were found.

Washing the petroleum-ether soluble material five times with distilled water showed a loss of 8 mg. of material by weight, which is 11.8 per cent of the total petroleum-ether soluble material.

The one patient with hypercholesterolemia did not show a marked difference in lipid values with the two methods of extraction.

On the basis of all the total lipid values given for normals, as done by the alcohol-ether extraction method by all the accepted authorities, our values for the blood dyscrasias have been high. Our values in the above series with the petroleum-ether extracts average 60.6 per cent of the values obtained with the alcohol-ether extracts, and are higher than normal.

It is obvious on the basis of the findings of Christensen, and Van Slyke and Folch, whose findings we now confirm, that a new standard of normal total blood lipid values must be determined. We are now investigating this question and we shall report our observations on it later.

REFERENCES

1. Pernokis, Evans W., and Freeland, M. R.: Blood Chemistry Determinations in Pernicious Anemia, *J. LAB. & CLIN. MED.* 26: 1177, 1941.
2. Pernokis, Evans W., and Freeland, M. R.: Blood Chemistry Observations in Leucemias, *J. LAB. & CLIN. MED.* 26: 1310, 1941.
3. Christensen, H. N.: Contaminants of Blood Phospho-lipids, *J. Biol. Chem.* 129: 531, 1939.
4. Van Slyke, D. D., and Folch, J.: Nitrogenous Contaminants of Petroleum-Ether Extracts, *J. Biol. Chem.* 129: 539, 1939.

MEDICAL ILLUSTRATION

RUBBER CASTS OF MEDICAL SUBJECTS

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WAX formulas have been used for some time for casting lifelike subjects. Although they give a true representation of the subject, they are not as durable as might be desired. By substituting prevulcanized latex for the wax, a permanent cast may be obtained. Such rubber casts have many of the advantages of the wax cast, with few of the disadvantages. Fig. 1A is a photograph of a spleen chosen to illustrate this process. The subject is a poor one, since it was removed from a body that had been preserved for several months. However, it is adequate for this description. A fresh specimen would have been more desirable.

Before starting the actual molding, a cardboard or glass box is made to hold the model. Glass was used for the box seen in Fig. 1B and serves to illustrate better the process. A small amount of plaster is poured into the center of the box. The specimen is placed in the plaster and vibrated to rid the surface of bubbles. By pounding several times on the table near the box, these bubbles will disappear, provided the plaster is in a liquid state. Additional plaster is poured into the box and a brush is used to work the plaster up to the edge of the specimen (Fig. 1C). This edge serves as the line of separation previously determined.

After the plaster is set, it is removed from the box, and the spleen is then dislodged from the set plaster. The edges around the impression of the spleen are trimmed of loose plaster and particles that are likely to break off. This set plaster form constitutes the lower half of the mold. Registration grooves are then cut in each corner, as seen in Fig. 1D. These are necessary to insure a perfect fit of the upper and lower halves. The edges of the lower half are greased with petroleum jelly so that it will separate readily from the upper half which is to be made, as shown in Fig. 1E. Care must be taken to prevent the petroleum jelly from getting on the surface of the plaster created by the spleen. If this happens, the surface ceases to absorb the water from the rubber which is to be poured later. The specimen is then replaced.

The plaster is mixed and the upper half of the mold is made, as shown in Fig. 1F. The molding material is applied with a brush into the crevices on the surface of the spleen. The procedure previously described should be followed to remove any air bubbles that may be present. After the plaster has set, the mold is opened and the specimen is removed. There are now two halves of the mold. Before these are put together for the making of the rubber positive,

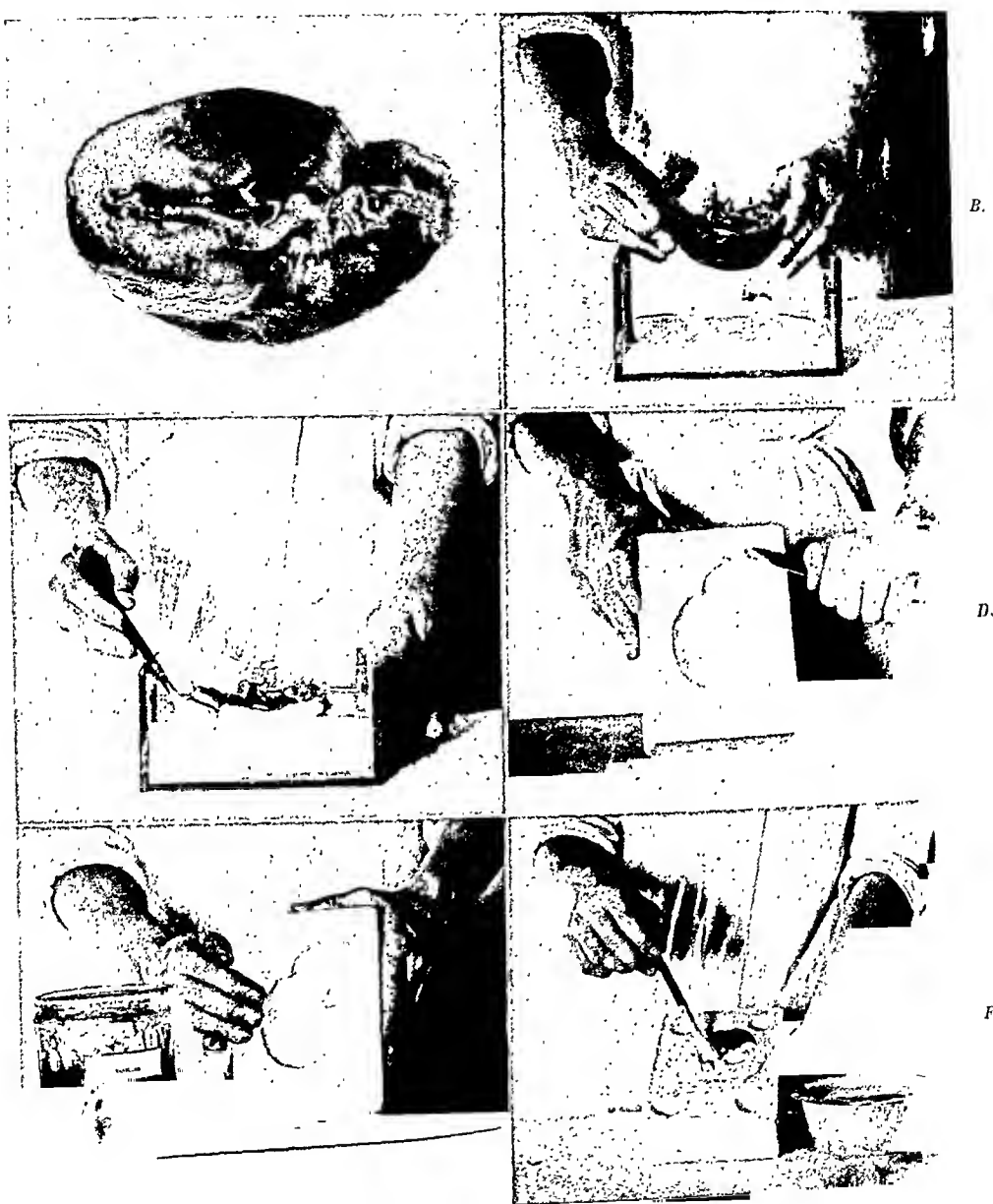


Fig. 1.—*A*, A preserved spleen.
B, The spleen is placed in a glass box containing a small amount of plaster.
C, The plaster is worked up to the edge of separation which has been determined previously.
D, Registration notches are cut in the set plaster.
E, Petroleum jelly is applied to the edge of the first half of the mold.
F, The specimen is replaced and the plaster applied to form the second half of the mold.

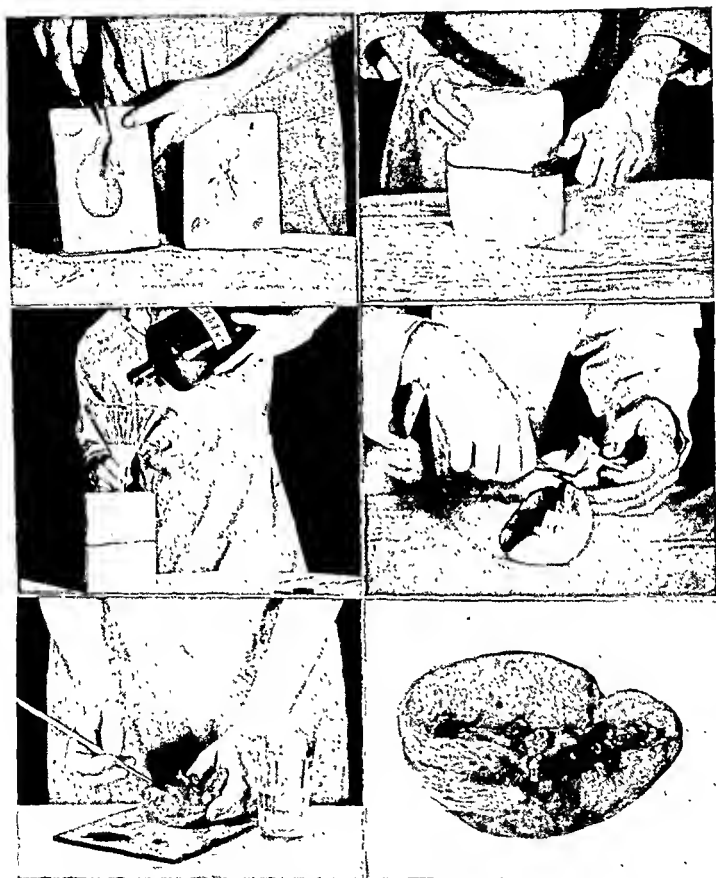


Fig. 2.—A, An opening is provided into which liquid rubber may be poured.
 B, The two halves of a mold are held together with wire or rubber bands.
 C, Liquid rubber is poured into the closed mold. After sufficient thickness is built up, the excess rubber is poured out.
 D, The rubber formed by the funnel-shaped opening is cut away.
 E, The specimen is colored to resemble a fresh spleen.
 F, The completed cast.

a funnel-shaped opening should be cut at the end that affords the best drainage, as illustrated in Fig. 2A. The two halves are placed together and held securely with wire or large rubber bands (Fig. 2B). Such rubber bands can be cut from an old inner tube and serve well for this purpose. The mold is then allowed to dry thoroughly. This usually takes several days; if gentle heat is applied, only a few hours are required to dry the mold.

Liquid vulcanized latex, which has been mixed with a mineral filler such as zinc oxide, is poured into the mold until it is filled completely (Fig. 2C). The mold is then allowed to set until the desired thickness is built up on the inside. As the dry plaster absorbs the water from the liquid rubber, more rubber is added to keep the mold filled at all times. When the cast has obtained the desired thickness, the excess rubber is poured out. This deposit is developed as a crust next to the plaster mold. The thickness of the crust depends on the dryness of the plaster and the consistency of the rubber-filler mixture. For those persons familiar with casting in terra cotta with clay slips, it should be mentioned that the rubber cast is developed within the plaster mold in a similar manner.

When the desired thickness of the rubber has been built up within the mold and the excess latex has been removed, the cast within the mold is allowed to dry overnight. Gentle heat speeds up this process and at the same time dries the plaster mold, thus making it more suitable for casting a second impression. The mold is then opened and the cast is removed. The part created by the funnel-shaped opening is cut away, as seen in Fig. 2D. Unless a nonmetallie water color, having a base reaction, is added to the liquid rubber before it is poured, the resultant cast will be cream colored. Any material having an acid reaction causes the rubber to coagulate and makes it unfit for use. Furthermore, rubber should not be mixed in bowls or containers made of copper or zinc, since these metals destroy the elasticity of the rubber and prevent it from setting properly. After the cast has set within the mold, it is removed. Any trimming or correction of defects that may be necessary should be done with fine-pointed scissors, a razor blade, or a sharp knife that has been dipped into water. The water causes these instruments to cut the rubber without dragging or leaving any rough spots. Small defects are removed with sandpaper, and the cast is given a coat of white shellac or lacquer to prepare it for coloring.

The transparent oil colors used for photo tinting serve excellently for coloring such casts, as illustrated in Fig. 2E. After the color is dry, the cast is given a coat of dammar varnish to make it resemble more closely the wet fresh specimen.

It is absolutely necessary that the color be dry before applying the varnish. Fig. 2F shows the completed rubber spleen. This may be compared with the original seen in Fig. 1A.

The specimen can be mounted in various ways. A base may be attached to the specimen at the point of the funnel-shaped opening. A thin metal rod is generally forced through this opening and sealed within the cast. The other end of the rod is anchored through a wooden or cast metal base.

The liquid vulcanized latex used for this purpose may be purchased from the General Latex and Chemical Co., Cambridge, Mass.; which firm sells the rubber already mixed with the filler. The latex may also be bought for mixing with any filler for specific purposes. This company is not interested in small orders and refers the prospective buyer to one of its agents who sells the vulcanized latex or one of the mixtures under a trade name at higher prices. In gallon lots the price should not exceed fifty cents a pint. However, it is sold under various trade names for as much as three dollars a pint. The average completed specimen would contain only three or four ounces of rubber. However, as much as two quarts may be necessary to fill the mold in order to get the four ounce deposit. The rubber which is poured from the mold may be used again.

The technical process is slightly different with each specimen that presents its own problems. For example, openings, air vents, and drainage vents must be placed at different points, according to the specimen that is being molded. Therefore, this description will overlap at points and be repeated.

The heart is a more complicated subject. A fresh specimen is easier to work with and gives better results than the preserved specimen used in this case. The wall to confine the plaster is made of cardboard (Fig. 3A). The plaster is poured into this space and worked up to the line of separation which has been determined previously. After the plaster has set and formed half of the mold, the specimen is removed (Fig. 3B). The edges are then trimmed and registration grooves are cut. This is done so that the other half of the mold, which is to be made, will fit perfectly with the first half.

After the registration grooves are cut, the first half of the mold is greased with petroleum jelly (Fig. 3C) to make separation possible. Care must be taken to prevent the petroleum jelly from touching the area to be covered with rubber, since the jelly prevents absorption of the water from the rubber into the plaster.

The specimen is then replaced in the mold, and the other half is made (Fig. 3D) by applying the plaster-water mixture directly to the specimen, thus covering the specimen and the first half of the mold.

In Fig. 3E are shown the two halves of the mold with the specimen in-cased. On one-half of the mold an area is outlined in pencil to form the entrance for a funnel through which the rubber is to be poured (Fig. 3F). Notice that this penciled area extends into two parts of the specimen, one at the base of the heart and the other at the cut section of the descending aorta. This allows the liquid rubber to drain from both parts of the specimen. The penciled area is cut out of the plaster to permit the insertion of the funnel seen in Fig. 4C. These areas also serve to drain the rubber from the mold in creating a hollow cast; therefore, they are cut into areas that are likely to form pockets. Pockets without openings will prevent the rubber from flowing out by gravity.

The two halves of the mold are wired together or held together with large rubber bands cut from sections of old automobile inner tubes (Fig. 4B). After the mold is thoroughly dry, the liquid vulcanized latex is poured through the

opening (Fig. 4C). The mold is then allowed to set until a sufficient thickness is built up by the absorption of the water from the liquid rubber into the mold. If the plaster is thoroughly dry, this takes only a few minutes; if the mold is wet, it may take hours. After a cast of sufficient thickness has been formed, the excess rubber is poured out and the hollow specimen is allowed to set overnight to strengthen the rubber. Gentle heat hastens this process and has a

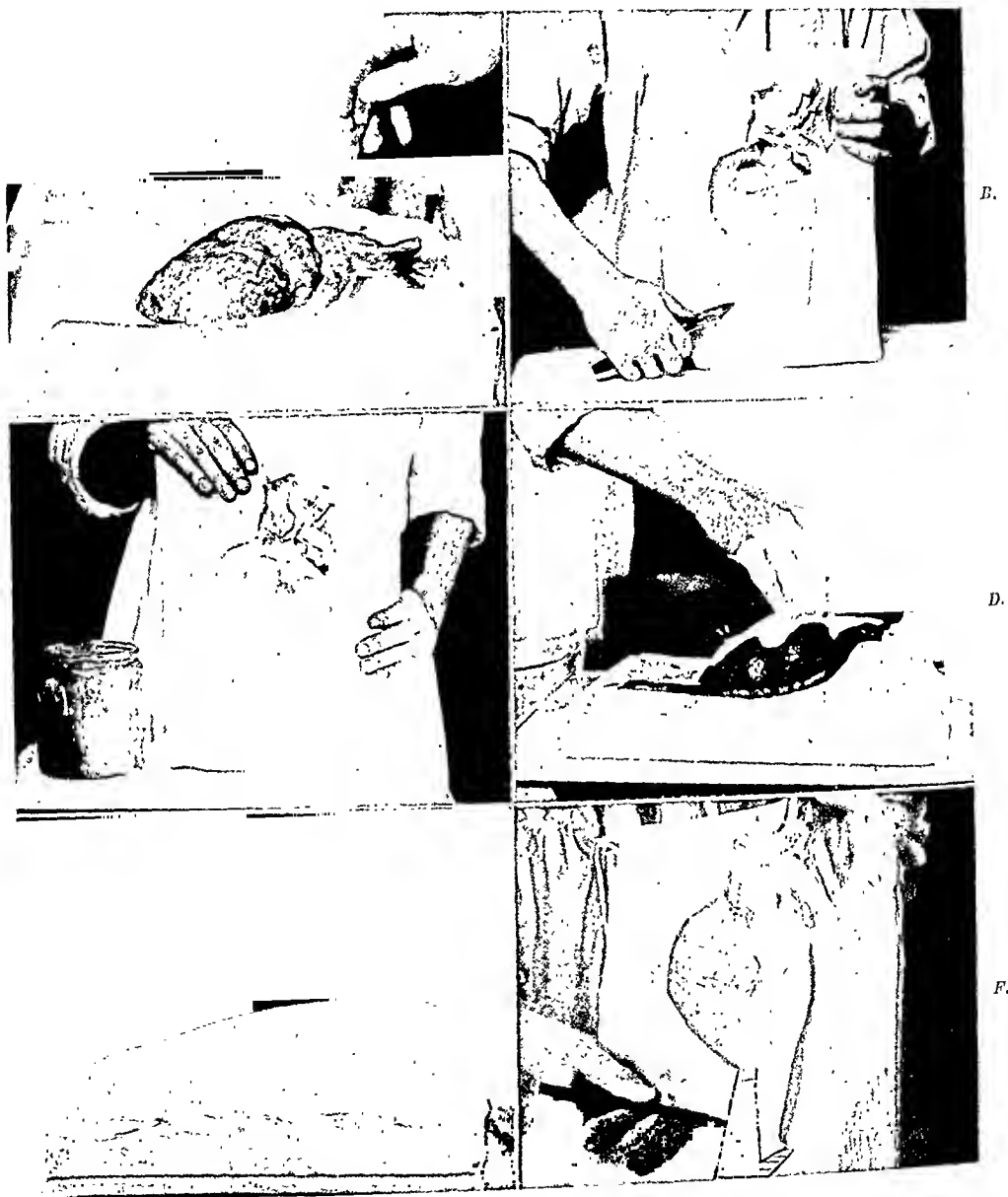


Fig. 3.—A, Making the first half of the mold.
 B, Cutting registration grooves.
 C, Petroleum jelly applied as a separating medium.
 D, Making the second half of the mold.
 E, The two halves of the mold with the specimen incased.
 F, Marking the area to be cut away for pouring the rubber.

beneficial effect on both the mold and the cast. When the specimen is thoroughly dry, the mold is opened and the cast is removed (Fig. 4D). This positive impression will probably tear if the mold is opened too soon after the pouring.

The rough edge formed by the seam in the mold is trimmed off with a razor blade and sharp knives (Fig. 4E), which, if dipped into water, glide through

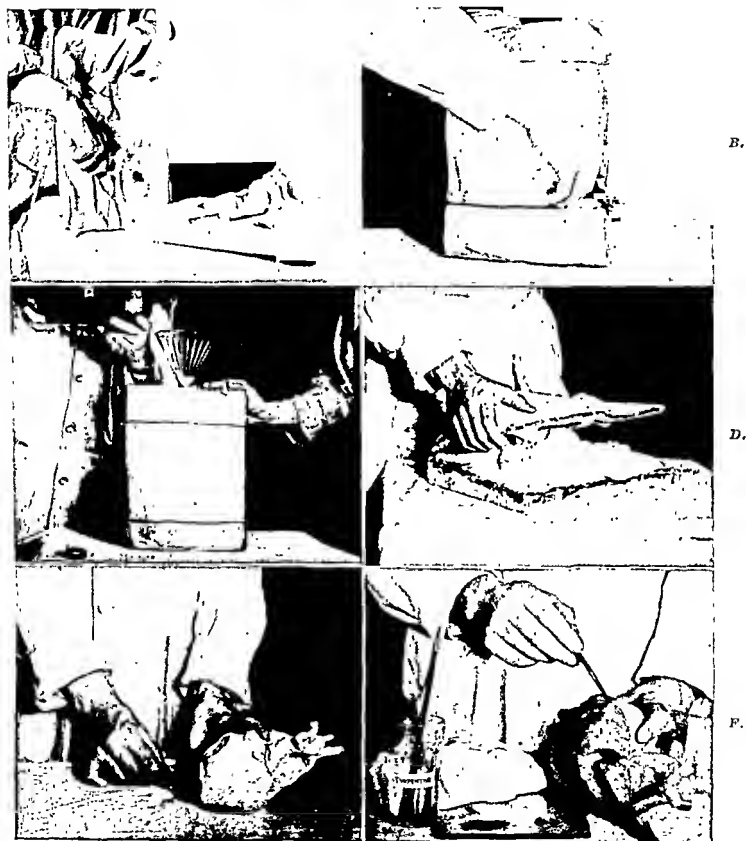


Fig. 4.—A, Cutting away the opening for pouring the rubber.

B, Wiring the mold together.

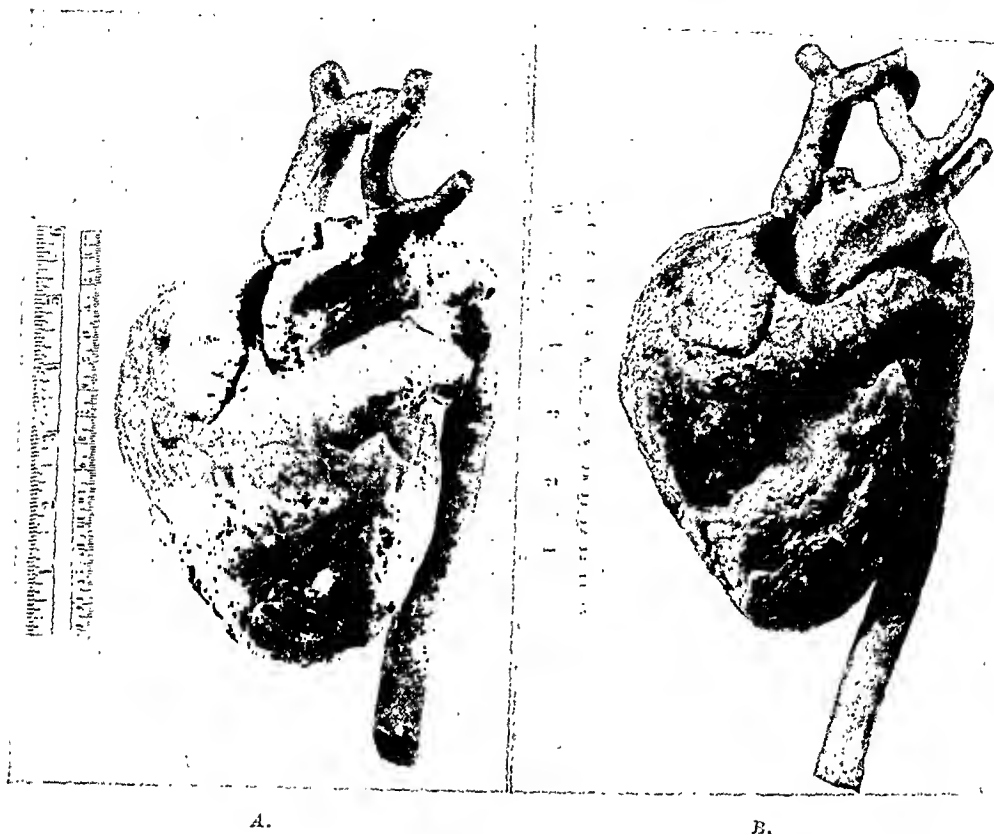
C, Pouring the rubber into the mold.

D, Separating the two halves of the mold.

E, Trimming away the edge formed by the line of separation.

F, Coloring the rubber cast.

the rubber more easily. Any rough areas remaining may be sandpapered smooth or worked smooth with emery wheels, wire brushes, and polishing devices, after which the specimen is given a coat of shellac.



A.

B.

Fig. 5.—A, The actual preserved heart. B, The rubber cast of the same heart colored to resemble a fresh specimen.

Coloring is then applied to simulate either a fresh or a preserved specimen (Fig. 4F). This is done with transparent oil paints or tempera colors. After completely drying, the specimen is given a coat of dammar varnish to create the glossy effect of a fresh specimen. It is then mounted.

Fig. 5A is a photograph of the actual preserved specimen. Fig. 5B is the rubber cast of the specimen. The slight difference in tonal value is the result of applying more red tints to the rubber cast to obtain the appearance of a fresh specimen.

REFERENCE

1. Clarke, C. D.: Molding and Casting, Its Technique and Application, Baltimore, 1938, John D. Lucas Co.

DEPARTMENT OF ABSTRACTS AND REVIEWS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

SCURVY, Human Experimental, Lund, C. C., and Crandon, J. H. J. A. M. A. 116: 663, 1941.

A vitamin C free diet produced scurvy in one of the authors at the end of five months, and certain symptoms of it were severe at six months.

The ascorbic acid level of the blood plasma fell to zero in forty-two days and that of the white blood cells in one hundred and twenty-two days.

A wound healed well after the patient had been three months on a scorbutic diet but not after six months.

There is no correlation between vitamin C intake and plasma levels, and the incidence of postoperative atelectasis and pneumonia.

Vitamin C deficiency may be a factor in failure of some human wounds to heal.

A dietary history should be taken on all patients who come to major surgery and, if a long-continued marked deficiency of the intake of vitamin C is found, the patient should be given from 1 to 4 Gm. of ascorbic acid daily.

AMEBA, Non-Pathogenic, Clinical Symptoms Associated With So-Called, Rothman, M. M., and Epstein, H. J. J. A. M. A. 116: 694, 1941.

Stool specimens from 406 patients located in and about Philadelphia harbored one or more of the five forms of intestinal amoebas. The most prevalent was *Endolimax nana*; *E. coli* was second.

These organisms, including *Diadamoeba fragilis*, had a causal relationship to their symptoms to the extent of 44.1 per cent.

The most common symptoms were constitutional; i.e., extreme tiredness and weakness. Of local nature were abdominal discomfort and diarrhea.

When a coexisting disease is active, the results to be expected may be only partial; i.e., only those symptoms for which the amoeba is responsible will disappear.

The response to carbarsone therapy is usually within three to five days. When no improvement is noted by the tenth day, one may be fairly certain that the symptoms were not due to the presence of the amoeba.

From a clinical point of view, all forms of amoeba appear to have a pathogenic role. Furthermore, since they may represent a serious public health menace and problem, it is suggested that they should be accepted and treated as such until clinical evidence to the contrary can be proved.

SULFANILAMIDES, Method for Obtaining Rapid Bacterial Growth in Cultures From Patients Under Treatment With, Janeway, C. A. J. A. M. A. 116: 941, 1941.

The authors recommend the addition of para-aminobenzoic acid to a final concentration of 5 mg. per hundred centimeters to all routine culture media. It will make cultures from patients under treatment with the sulfonamide drugs mean what they should—i.e., the presence or absence of viable organisms. By way of caution, it must be noted that the body fluids may frequently be sterilized by sulfonamide therapy and then later become positive again when the patient relapses. During the remission positive cultures could be obtained only from the local focus, and no amount of para-aminobenzoic acid would make the cultures of the body fluids positive.

PROTHROMBIN: Quick's Test Simplified by the Use of a Stable Thromboplastin, Souter, A. W., and Kark, R. Am. J. M. Sc. 200: 603, 1940.

Brains were removed from freshly-killed rabbits, and the larger superficial blood vessels were stripped off after washing with water. The brains were then ground to a paste, spread thinly on a glass plate, and thoroughly dried in an oven at 37° C. The dried residue was scraped off the plate, and about 15 c.c. of saline extract were prepared by adding 30 c.c. of 0.85 per cent sodium chloride solution to 3 Gm. of dried brain, mixing with a glass rod and incubating at 56° C. for fifteen minutes. On centrifuging the mixture at 2,000 r.p.m. for five minutes, a somewhat opalescent supernatant fluid was obtained. About 2 c.c. of this extract were set aside for testing as a control, and the remainder was placed in small serum bottles, 1 c.c. in each. The contents of these bottles were then "lyophilized," and at the completion of the process the evacuated bottles were set aside for storage at room temperature (about 20° C.).

The portion of extract which was kept as a control was tested for thromboplastic activity by using to determine the Quick prothrombin times of a series of dilutions of normal citrated plasma with prothrombin-free plasma prepared by the method of Kark and Lozner, so that the preparations contained 100, 80, 60, 40, 20, and 0 per cent prothrombin.

The technique used in the determination of these prothrombin times was essentially that of Quick. One-tenth cubic centimeter of thromboplastin was pipetted into a tube 10 by 75 mm., and the tube was placed in a constant temperature (37.8° C.) water bath. After a few minutes 0.1 c.c. of plasma was added, and immediately thereafter 0.1 c.c. of 0.277 per cent solution of calcium chloride. A stopwatch was started at the moment of delivery of the calcium chloride solution from the pipette. The tube was agitated gently in the bath for fifteen seconds and then rocked while held in the air until the contents ceased to flow freely. At this point the watch was stopped and the time was taken.

Bottles containing the dry, powdered, "lyophilized" material were opened at intervals of one, two, three, four, and ten weeks after "lyophilization," and the contents were restored to their original volume by the addition of distilled water, when a somewhat opalescent solution was readily obtained. The thromboplastic activity was then determined on each occasion by testing against plasmas containing various percentages of prothrombin. At the same time the results obtained were compared with those given by an extract freshly made up from the original dried rabbit brain substance stored in the icebox at 5° C. The figures for these results are shown in Table I.

TABLE I

QUICK PROTHROMBIN TIME OF MIXTURES OF NORMAL AND PROTHROMBIN-FREE PLASMAS,
USING FRESHLY PREPARED AND LYOPHILIZED THROMBOPLASTINS

	Time in weeks after lyophilization of throm- boplastin at which Quick prothrombin times were observed	Quick prothrombin times of mixtures of normal plasma and prothrombin-free plasma (seconds) Percentage of normal plasma in mixture					
		100	80	60	40	20	0
Fresh thromboplastin extract for lyophilization	Control	21	23	26	29	40	No clot
"Lyophilized" thromboplastin	1	21	24	27	32	50	No clot
Freshly prepared thromboplastin		20	21	23	28	42	No clot
"Lyophilized" thromboplastin	2	24	26	28	34	56	No clot
Freshly prepared thromboplastin		23	25	26	32	52	No clot
"Lyophilized" thromboplastin	3	23	25	28	34	54	No clot
Freshly prepared thromboplastin		21	23	25	29	45	No clot
"Lyophilized" thromboplastin	4	21	24	26	31	49	No clot
Freshly prepared thromboplastin		20	22	25	30	40	No clot
"Lyophilized" thromboplastin	10	21	23	25	33	68	No clot
Freshly prepared thromboplastin		21	23	26	33	68	No clot

STREPTOCOCCUS, Convalescent Serums (Scarlatinal), Platon, E. S., Dwan, P. F., and Hoyt, R. E. *J. A. M. A.* 116: 11, 1941.

The clinical and experimental evidence presented suggests the following points:

1. Erythrogenic toxin, produced in varying amounts by any of Griffith's thirty types of hemolytic streptococci, is important in the pathogenesis of scarlet fever and other streptococcal infections, and its prompt neutralization is desirable.
2. Other toxic products of the hemolytic streptococcus, such as leukocidin, fibrinolysin, and spreading and invasive factors, have received inadequate consideration.
3. The ideal antiserum for treatment of infections due to group A hemolytic streptococci (including scarlet fever) is one which is rich in both group specific and type-specific antibodies and which is homologous.
4. Though commercial scarlet fever antitoxin has merit as a neutralizing agent against erythrogenic toxin, it lacks any antibacterial or anti-invasive properties.
5. Pooled convalescent scarlet fever serum in large doses confers considerable antitoxic immunity and in addition an effective antibacterial immunity.
6. Heterologous serum (horse) may cause reactions which can predispose to bacterial spread.
7. Attempts to increase the antitoxic titers of human convalescent donors have been unsuccessful.
8. Hyperimmunization of convalescent donors with streptococcus vaccines did not result in increased antibacterial activity under the conditions of the authors' experiment.
9. Pooling human sera according to antibody activity and type and administration of this product intravenously offer an immunologically sound basis for the serum treatment of group A streptococcus infections.

TISSUE: A Rapid Method of Staining Fat in Frozen Sections With Osmic Acid, Krafiian, A. *Arch. Path.* 50: 766, 1940.

1. Fix tissue in a 10 per cent solution of formaldehyde for twenty-four hours.
2. Cut frozen sections 10 microns thick.
3. Bring to a boil a 1 per cent aqueous solution of osmic acid in a pyrex test tube and pour the solution into a small Stender dish.
4. Place the section in the hot osmic acid solution and keep it in a paraffin oven at 60° C. for five minutes.
5. Wash in a basin of tap water.
6. Counterstain in 1 per cent aqueous eosin or phloxin for one minute.
7. Wash rapidly in tap water.
8. Transfer the section to a slide and let drain off.
9. Place 3 large drops of glycerin jelly (previously melted in a paraffin oven or a water bath) and cover with a cover slip.

Result: The fat globules are black or gray black; the background is red.

For emergency examination, bring a 10 per cent solution of formaldehyde to boil in a pyrex test tube (60 c.c. capacity); drop a thin piece of biopsy or autopsy material into it, and place the specimen in a hot oven (60° to 65° C.) for ten minutes. Cut thin frozen sections and stain by the method described.

It is not necessary to discard the used osmic acid solution. Pour it back into the stock bottle and use it over and over.

TISSUE, Combined Stain for Fat and Elastic, French, O. *Arch. Path.* 50: 1245, 1940.

Method of Embedding

1. Fix tissues in solution of formaldehyde for twenty-four hours or longer.
2. Wash in running water for five hours.
3. Place in a 5 per cent aqueous solution of gelatin (Knox) at 37° C. for twelve to twenty-four hours.

4. Place in 10 per cent aqueous solution of gelatin at 37° C. for six to twelve hours.
5. Embed in 10 per cent gelatin. Harden in icebox for two to four hours.
6. Before cutting, place individual blocks in solution of formaldehyde for twelve to twenty-four hours (either blocks or sections may be preserved indefinitely in solution of formaldehyde).
7. Cut sections on freezing microtome at 10 microns.
8. Before staining, place sections in formaldehyde for twelve to twenty-four hours.

Method of Staining

1. Rinse section well in distilled water.
2. Dip several times into 70 per cent alcohol.
3. Place in fat and elastic tissue stain, warmed to not more than 45° C. for fifteen minutes.

Formula for stain:

Weigert's elastic tissue stain (ripened)	10 c.c.
Distilled water	2 c.c.
Scarlet red, saturated solution in equal parts of acetone and 70 per cent alcohol	8 c.c.

4. Rinse in 70 per cent alcohol.
5. Rinse in distilled water.
6. Place in Delafield's (or other) hematoxylin for one to two minutes.
7. Rinse in distilled water.
8. Differentiate in 0.5 per cent acid alcohol if desired.
9. Immerse in weak ammonia until the section becomes blue.
10. Rinse in distilled water.
11. Transfer to a slide, drain, and mount in glythrogel. (If desired, 1 per cent aqueous vert lumiere may be used as a counterstain after the hematoxylin, followed by mild decolorization in 60 per cent alcohol.)

DEXTROSE TOLERANCE, Oral and Intravenous, in Cases of Acute (Catarrhal) Jaundice,
Paehman, D. J. Am. J. Dis. Child. 60: 1277, 1940.

The tolerance to dextrose (orally and intravenously administered), the icteric index, and the bilirubin content of the blood were determined serially in 12 children with catarrhal jaundice.

The icteric index closely paralleled the serum bilirubin content of the blood.

Total cholesterol values were normal or below normal in all but 3 patients. The percentage of esters was decreased in 75 per cent of the children.

No correlation was found between the degree of icterus and the type of dextrose tolerance curve obtained.

Decreased tolerance to orally administered dextrose was frequently found when the response to the previous intravenous test had been normal.

When the intravenous test showed a decrease in tolerance, the tolerance to orally administered dextrose was also decreased.

Improvement of a previously decreased tolerance to dextrose (orally or intravenously administered) usually accompanied clinical improvement and a decrease in the degree of icterus.

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